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THE EFFECT OF CERTAIN ADDED MATERIALS ON BORDEAUX MIXTURE IN THE CONTROL OF PEACH BLIGHT AND LEAF CURL[1]

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INTRODUCTION

IN THE STANDARD method for controlling peach blight, caused by *Coryneum Beijerinckii* Oud., bordeaux mixture (10–10–100) is applied to the trees after all leaves are off in the autumn, but before the winter rains begin. Since this is the only application given to protect the twigs against blight during the following three or four months, when heavy and prolonged rains occur, and since this application is also expected to prevent leaf curl caused by *Taphrina deformans*, success of the control is largely dependent upon resistance of the fungicide deposit to dissipation by atmospheric agencies.

In earlier trials (*12*),[3] bordeaux to which 4 per cent of a dormant petroleum-oil emulsion was added, proved more weather-resistant than bordeaux without oil—results that were in agreement with those of Winston, Bowman, and Yothers (*14*). In 1936–37 and 1937–38, therefore, further trials were undertaken to determine whether smaller amounts of petroleum oil would reduce the loss of bordeaux from peach twigs as effectively as 4 per cent does, and whether other materials such as cottonseed oil and bentonite were useful in this respect.

The effect of petroleum oil on the toxicity of bordeaux to spores of *Coryneum Beijerinckii* (*13*), and on the spray's "retention"[4] and "coverage" qualities were also studied.

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[3] Italic numbers in parentheses refer to "Literature Cited" at the end of this paper.

[4] The literature is not unanimous regarding designation of the qualities that determine the efficacy of insecticides and fungicides. Thus the amount of spray per unit of surface remaining after application, is called "initial spray deposit" (*16*), "deposition" (*8*), "initial adhesiveness" (*9*), and "retention" (*2*); the distribution of the material over the surface is "coverage" (*3*); and the resistance to weathering is "adherence" (*10*) or "tenacity" (*2*). Horsfall, Heuberger, Sharvelle, and Hamilton (*8*) designate as "fungicidal value" that ability of the material to prevent spore germination; but the present writer prefers "toxicity" or "toxicological value."

EFFECT OF PETROLEUM OIL, COTTONSEED OIL, AND BENTONITE ON TENACITY OF BORDEAUX

Each spray treatment was given in the autumn to four randomized plots, each containing four Paloro peach trees of fairly uniform size. The treatments were bordeaux mixture (10–10–100) to which had been added different amounts of petroleum-oil emulsion, tank-mix petroleum oil, cottonseed oil, or bentonite. Emulsion A was a flowable-type emulsion containing 80 per cent, by weight, of a petroleum oil of 102 seconds Saybolt viscosity and 70 per cent unsulfonated residue. Tank-mix oil was a petroleum oil similar to that in emulsion A emulsified with blood albumin just before it was added to the spray tank. Emulsion B was a paste-type emulsion containing 82 per cent, by weight, of a petroleum oil of 96 seconds viscosity and 94 per cent unsulfonated residue. The cottonseed oil was a commercial grade. The bentonite was a natural product containing some magnesium oxide.

As soon as the spray dried, 200- to 250-gram samples of twigs produced during the past summer were collected and weighed. The twigs were then cut into convenient lengths, placed in glass jars, and shaken for 10 minutes with 500 cc of nitric acid water (20 cc nitric acid, of 1.42 sp. gr., per liter). The wash water was filtered and tested for copper by the sodium diethyl dithiocarbamate method[5] that Callan and Henderson (*1*) described.

Other collections were made after several inches of rain had fallen (December or January) and again just before the buds swelled in the spring (February). The amounts of copper on these samples were the basis for determining the weather resistance or tenacity of the bordeaux.

Experiments of 1936–37.—Sprays were applied November 20, 1936, well before the first major wave of twig infection by *Coryneum* which was initiated during rains falling between December 20 and 28. The season was marked by recurrent attacks of the disease during January, February, and March—particularly severe being a wave initiated in early March.

According to the November 20 analyses (table 1), considerable variability existed in the initial amount of copper deposited by the various treatments. These data, together with those secured in other years, will be discussed later.

[5] In the present work the results obtained by the Callan and Henderson method were consistently higher, by about 3 per cent, than results obtained by the electrometric method, but compared more favorably with those secured by the iodometric method. Though the last-named method cannot be used when very small amounts of copper are to be determined, it is a valuable referee for standards used in colorimetric tests.

TABLE 1

EFFECT OF PETROLEUM OIL AND BENTONITE ON TENACITY OF BORDEAUX DEPOSIT AND ON CONTROL OF PEACH BLIGHT, 1936–37

Amount and type of material added to bordeaux (10–10–100)*	Deposit November 20, milligrams copper on 100 grams of twigs	Analyses, January 6†		Analyses, February 23‡		Disease, March 11–14§		Disease, April 26–29§		
		Milligrams copper on 100 grams of twigs	Per cent of copper lost	Milligrams copper on 100 grams of twigs	Per cent of copper lost	Per cent of twigs infected	Average number of lesions on 100 twigs	Per cent of twigs infected	Average number of lesions on 100 twigs	Per cent of twigs killed by the disease
None	28.9	10.7	62.0	2.6	90.9	12	15	61	104	14
1 per cent of petroleum emulsion A	18.4	6.8	62.9	2.0	88.6	26	30	73	241	26
2 per cent of petroleum emulsion A	24.9	12.5	49.4	3.8	84.3	7	9	38	52	7
3 per cent of petroleum emulsion A	20.4	13.3	34.6	3.8	81.6	7	9	68	133	13
4 per cent of petroleum emulsion A	40.9	29.9	27.3	11.5	71.9	2	2	19	27	4
1 per cent of petroleum emulsion B	16.6	5.9	63.9	1.7	89.0	10	12	74	143	18
2 per cent of petroleum emulsion B	34.4	20.0	41.7	7.8	77.8	4	4	18	27	3
0.6 pound of bentonite per 100 gallons	24.3	10.7	55.7	2.6	88.1	8	11	64	117	10
2 pounds of bentonite per 100 gallons	23.6	9.8	53.3	2.2	89.8	11	15	50	87	11
Difference required for significance¶ 19:1 odds	....	....	9.56	....	5.70	4.04	6.03	9.08	33.62	4.83
Difference required for significance¶ 99:1 odds	....	....	12.95	....	7.72	5.48	8.17	12.31	45.56	6.54
Calculated F value‖	....	....	15.94	....	11.17	24.62	15.11	4.90	33.94	18.37

* Bordeaux made with quicklime.

† Analyses made after 2.73 inches of rainfall.

‡ Analyses made after 11.83 inches of rainfall.

§ On March 11–14 in unsprayed trees the percentage of twigs infected was 94, and the average number of lesions per 100 twigs was 387. On April 26–29 these values were 99 and 1,460, respectively, and the percentage of twigs killed was 46.

¶ The differences required for significance at odds of 19:1 and 99:1 were obtained by multiplying the standard error of the difference by t values of 2.145 and 2.977 respectively.

‖ All these ratios exceed the F value for the 1 per cent point.

After 2.73 inches of rain had fallen, analyses (January 6) revealed marked differences in rate of copper loss from trees receiving the various treatments. Apparently neither of the two types of oil (emulsion A and emulsion B) when used at the rate of 1 per cent influenced the tenacity of bordeaux. The February 23 analyses further emphasize this point, the percentage of copper lost being practically the same for bordeaux alone, bordeaux with 1 per cent of emulsion A, and bordeaux with 1 per cent of emulsion B. Neither in the January 6 nor in the February 23 analyses was bentonite found significantly to reduce the loss of copper.

Increasing the amounts of oil emulsion above the 1 per cent resulted in successively improved tenacity. In this respect, 4 per cent of emulsion A was significantly more efficient than 2 or even 3 per cent, and 2 per cent of emulsion B produced greater tenacity than 1 per cent.

January 6 analyses showed that most treatments still retained considerable amounts of copper. Exceptions were bordeaux plus 1 per cent of emulsion A and bordeaux plus 1 per cent of emulsion B, where the low amount of residue was influenced by low initial deposits. The depletion of the spray coating on trees receiving these two treatments is reflected in the control data obtained March 11–14. Bordeaux plus 1 per cent of emulsion A, in particular, was showing signs of losing its effectiveness at this time. The possibility that the addition of oil might have affected the initial deposit will be discussed later.

According to data on disease conditions in unsprayed trees, as given in the footnote of table 1, most other treatments gave good control up to March 11–14. During early March, however, rains initiated abundant infection; the resulting lesions appeared after March 14, and between this date and April 26–29 the number of lesions on unsprayed trees more than tripled. Bordeaux and bordeaux plus 1 per cent of emulsion A or of B gave poor control during this period, whereas bordeaux plus 2 or 4 per cent of emulsion A and bordeaux plus 2 per cent of emulsion B proved much more efficient. The poor control obtained with bordeaux plus 3 per cent of emulsion A cannot be explained fully by the data at hand. The low initial deposit may have been responsible to some extent, though the amount of copper remaining on February 23 was the same as that for 2 per cent of emulsion A. The 3 per cent of emulsion treatment controlled well up to the early March infection period, but failed thereafter.

Bentonite did not significantly influence the control one way or the other.

On February 1, 1937, bordeaux with and without oil was applied to almond trees. As no blight of consequence developed in this orchard,

TABLE 2

RELATION OF PETROLEUM OIL EMULSION TO THE RETENTION AND TENACITY OF BORDEAUX DEPOSIT ON ALMOND TWIGS, 1937

Amount of oil added to bordeaux (10–10–100)	Analyses, February 1; milligrams of copper per 100 grams of twigs*	Analyses, February 14; milligrams of copper per 100 grams of twigs†	Per cent of copper lost
None	22.1	6.4	71.1
1 per cent of emulsion A	24.1	4.7	81.5
3 per cent of emulsion A	30.0	16.9	44.7
Difference required for significance:‡ 19:1 odds	3.65	3.90	12.7
Difference required for significance:‡ 99:1 odds	5.24	5.60	18.3
Calculated F value§	12.85	29.43	23.58

* Spray was applied February 1 and samples for these analyses were made immediately after it dried.
† Between February 1 and February 14, 5.19 inches of rain fell.
‡ The differences required for significance at odds of 19:1 and 99:1 were obtained by multiplying the standard error of the difference by t values of 2.262 and 3.250 respectively.
§ All these ratios exceed the F value for the 1 per cent point.

TABLE 3

EFFECT OF DIFFERENT SUPPLEMENTS ON THE CONTROL OF PEACH LEAF CURL BY BORDEAUX MIXTURE

Amount and type of material added to bordeaux (10–10–100), 1936–37*	Per cent of leaves diseased†	Amount and type of material added to bordeaux (10–10–100), 1937–38*	Per cent of leaves diseased†
Unsprayed	56	Unsprayed	49
None	2	None	0.4
1 per cent of petroleum emulsion A	8	1 per cent of petroleum emulsion A	2
2 per cent of petroleum emulsion A	0.4	3 per cent of petroleum emulsion A	0.2
3 per cent of petroleum emulsion A	2	1 per cent of petroleum tank-mix oil	1
4 per cent of petroleum emulsion A	0.4	3 per cent of petroleum tank-mix oil	0.1
1 per cent of petroleum emulsion B	2	1 per cent of cottonseed oil	1
2 per cent of petroleum emulsion B	0.5		
0.6 pound of bentonite per 100 gallons	0.7		
2 pounds of bentonite per 100 gallons	0.5		

* Sprays were applied in 1936–37 on November 20; in 1937–38 on November 23.
† Observations on leaf infection made in late April. The percentage of leaves infected was determined by counting the leaves on 20 randomly selected twigs in each tree.

evidence on control was not obtained. The data on copper analyses (table 2), however, further confirmed the results secured on peaches, in that 1 per cent of oil emulsion had no effect on bordeaux tenacity, whereas 3 per cent materially increased it.

As peach-leaf curl developed abundantly on unsprayed trees, counts were made on the percentage of leaves diseased in trees receiving the treatments listed in table 1. The results (table 3) showed no marked failure of any treatment to control this disease, though in one of the

TABLE 4

EFFECT OF PETROLEUM AND COTTONSEED OILS ON TENACITY OF BORDEAUX DEPOSIT AND ON CONTROL OF PEACH BLIGHT, 1937–38

Amount and type of oil added to bordeaux (10–10–100)*	Deposit November 23, milligrams copper on 100 grams of twigs	Analyses, December 24†		Analyses, February 7‡		Disease development, May 4–5§		
		Milligrams copper on 100 grams of twigs	Per cent of copper lost	Milligrams copper on 100 grams of twigs	Per cent of copper lost	Per cent of twigs infected	Average number of lesions on 100 twigs	Per cent of twigs killed by the disease
None	36.0	23.1	35.8	9.6	73.7	76	176	10
1 per cent of petroleum emulsion A	23.9	14.5	39.4	7.4	69.3	77	190	13
3 per cent of petroleum emulsion A	26.5	21.8	18.8	12.9	49.4	75	153	7
1 per cent of petroleum tank-mix oil	31.2	24.0	23.0	7.3	75.9	78	195	12
3 per cent of petroleum tank-mix oil	32.1	27.5	13.3	13.7	57.5	69	129	8
1 per cent of cottonseed oil	28.7	19.1	33.5	11.2	60.9	70	155	7
Difference required for significance¶ 19:1 odds	....	....	12.96	....	13.30	11.70	62.27	4.84
Difference required for significance¶ 99:1 odds	....	....	17.92	....	18.40	16.18	86.11	6.69
Calculated F values	....	....	5.18‖	....	5.44‖	0.82	1.49	2.33

* Sprayed November 23. Bordeaux made with quicklime.

† Analyses made after 4.4 inches of rainfall.

‡ Analyses made after 10.75 inches of rainfall.

§ Infection before spray was applied resulted in 46 per cent of twigs becoming diseased with an average of 146 lesions per 100 twigs. On May 4–5 the percentage of twigs infected, average number of lesions per 100 twigs, and percentage of twigs killed in unsprayed trees were 99, 1278, and 43, respectively.

¶ The differences required for significance at odds of 19:1 and 99:1 were obtained by multiplying the standard error of the difference by t values of 2.131 and 2.947 respectively.

‖ Exceeds F value for the 1 per cent point.

four replications of bordeaux plus 1 per cent of emulsion A the incidence of the disease was rather high.

Experiments of 1937–38.—Twig infection was initiated in mid-November, 1937, and in consequence a considerable part of the disease recorded in the final counts on May 4–5, 1938, was present in the trees when the sprays were applied on November 23. The major part of the twig lesions appeared during November and December; none appeared during January, and only a few in February. Therefore, disease development in this season differed markedly from that in 1936–37, when lesions were appearing throughout the winter and a particularly large number appeared in early March. It is apparent that the problem of control in 1937–38 also differed from that in 1936–37, although the incidence of the disease was the same in both years (footnotes to tables 1 and 4).

Sprays were applied on November 23, and samples collected on the same day were analyzed for initial deposits (table 4). Collection of samples on December 24 (after 4.4 inches of rainfall) indicated, as in 1936–37, that 1 per cent of oil emulsion A did not affect bordeaux tenacity; but 3 per cent increased the tenacity considerably. The same general relation between these treatments and bordeaux without oil was maintained up to February 7, during which time an additional 6.35 inches of rain fell. According to the December 24 analyses 1 per cent of tank-mix oil appeared to favor bordeaux tenacity, but the February 7 analyses revealed no advantage over bordeaux without oil. Three per cent of tank-mix oil was about as efficient as 3 per cent of emulsion A in preventing copper loss. One per cent of cottonseed oil appeared, in the February 7 analyses, to have decreased the loss only slightly if any.

As was mentioned at the beginning of this section, twig lesions were developing in the trees at the time the sprays were applied. Two days after application an average of 146 lesions per 100 twigs were present on 46 per cent of the twigs. To show more clearly the control situation, the increment of 146 lesions per 100 twigs is deducted from the results in table 4 and is arranged with the data on copper residues as of February 7 (table 5). Some correlation between the amount of copper remaining on the twig and the number of new lesions is thus shown. The copper residues on this date have the same general relation to the percentage of twigs killed, although such differences cannot be considered statistically significant (table 4).

When, therefore, deductions are made for the disease developing before sprays were applied, all treatments are seen to give excellent control. The reason for this high efficiency is the manner in which disease

developed: the major attacks came early in the winter at a time when the spray film on the trees was new, instead of in late winter after weathering had reduced it, as in 1936–37.

TABLE 5

Relation of Amount of Copper Remaining on Twigs Near the End of the Season to the Control of Peach Blight Obtained with Bordeaux and Bordeaux plus Oil, 1937–38

Amount and type of material added to bordeaux (10–10–100)*	Milligrams of copper per 100 grams of twigs, February 7	Average number of lesions (per 100 twigs) developing after spraying†
Unsprayed	....	1,132
None	9.6	30
1 per cent of oil emulsion A	7.4	44
3 per cent of oil emulsion A	12.9	7
1 per cent of tank-mix oil	7.3	49
3 per cent of tank-mix oil	13.7	0

* Sprayed November 23. Bordeaux made with quicklime.

† Infection before the spray was applied on November 23 resulted in an average of 146 lesions per 100 twigs. The values in this column were obtained by deducting this number from the average number of lesions per 100 twigs reported in table 4.

TABLE 6

Improvement in the Tenacity of Bordeaux Deposit and the Control of Peach Blight When Petroleum-Oil Emulsion Was Added, 1937–38*

Treatment†	Deposit November 25, milligrams copper on 100 grams of twigs	February 27, milligrams copper on 100 grams of twigs	Per cent of copper lost	Per cent of twigs killed by the disease (April 15)
Bordeaux (10–10–100)	29.3	6.4	88.2	14
Bordeaux (10–10–100) plus 4 per cent of emulsion A	27.5	11.0	60.0	5

* From data secured in a commercial orchard.

† Spray applied November 25. Bordeaux was prepared with hydrated lime, which was soaked 1 to 2 hours before using.

Some additional studies were made in a commercial orchard where bordeaux (10–10–100) alone and bordeaux (10–10–100) plus 4 per cent of oil emulsion A were applied. These studies (table 6) further demonstrated the increased tenacity attending the addition of a large amount of oil to bordeaux. Some improvement in control was also apparent.

As in 1936–37, leaf curl developed abundantly in unsprayed plots in the experimental orchard. No significant difference in control attended the various treatments listed in table 4; all gave excellent protection (table 3).

EFFECT OF PETROLEUM-OIL EMULSION ON THE AMOUNT OF BORDEAUX DEPOSITED ON A SURFACE

The unsatisfactory control of twig infection in 1936 (table 1) which attended the applications of bordeaux containing 1 per cent of oil-emulsion A and of B was attributed, in part at least, to the low initial

TABLE 7

EFFECT OF PETROLEUM AND COTTONSEED OILS ON THE RETENTION OF BORDEAUX MIXTURE BY PEACH TWIGS

Amount and type of oil added to bordeaux (10–10–100)	Milligrams of copper on 100 grams of twigs		
	1936	1937	1938
None	28.9	33.3	20.1
None	....	29.6	....
None	....	36.0	....
1 per cent of emulsion A	18.4	23.9	....
2 per cent of emulsion A	24.9	....	....
3 per cent of emulsion A	20.4	26.5	20.1
4 per cent of emulsion A	40.9	....	....
1 per cent of emulsion B	16.6	....	....
2 per cent of emulsion B	34.4	....	....
1 per cent of tank-mix oil	....	31.2	....
3 per cent of tank-mix oil	....	32.4	30.2
1 per cent of cottonseed oil	....	28.9	24.9
2 per cent of cottonseed oil	....	....	24.6
Difference required for significance* 19:1 odds	6.17	6.85	6.50
Difference required for significance* 99:1 odds	8.40	9.28	8.98
Calculated F value	18.2†	2.76‡	3.74‡

* Differences required for significance at odds of 19:1 and 99:1 were obtained by multiplying the standard error of the difference by t values respectively as follows: 1936, 2.08 and 2.831; 1937, 2.064 and 2.797; 1938, 2.131 and 2.947.

† Exceeds F value for the 1 per cent point.

‡ Exceeds F value for the 5 per cent point.

deposit of the fungicide. A low deposit and unsatisfactory control were also recorded for the treatment containing 3 per cent of emulsion A: the amount of copper residue remaining on the trees in late February, shortly before the infection period that proved critical, was not materially lower than in treatments that remained effective during the critical period. On the other hand, treatments that controlled twig infection most satisfactorily—bordeaux containing 4 per cent of emulsion A and 2 per cent of emulsion B—gave initial deposits considerably higher than other treatments.

Whereas analysis of variance (table 7) shows that in 1936 the initial deposit of the bordeaux with 1 per cent of oil was significantly lower than that of bordeaux without oil, neither 1 per cent nor 3 per cent of

emulsion A significantly reduced deposits in 1937. In 1938, moreover, 3 per cent of emulsion A did not affect copper deposits. The treatment containing 3 per cent of tank-mix oil, on the contrary, deposited more copper than bordeaux without oil in 1938, but not in 1937. Considering these variations and the character of the results secured on almond in 1936 (table 2), at which time bordeaux plus 3 per cent of emulsion A deposited higher amounts of copper than bordeaux without oil, one can draw no definite conclusions regarding the effect of oil on initial deposits.

To obtain further information under laboratory conditions, a small spray applicator was constructed. This consisted of a framework at one end of which was attached a no. 16 De Vilbiss atomizer in a horizontal position; at the other end, 16 inches from the nozzle of the atomizer, a clamp held the object to be sprayed. A rubber tube led from the intake of the atomizer to a wide-mouthed glass jar holding the spray material. A small electric stirrer kept the spray material constantly agitated. The air blast to operate the atomizer was furnished by a laboratory pump equipped with an adjustable pressure-release valve. In order quickly to begin and end application without disturbing the delivery of spray from the atomizer, the nozzle was enclosed in a metal cup with an aperture in line with the stream of spray. This aperture was opened and closed by a shutter.

Though a number of improvements, such as that suggested by Horsfall, Heuberger, Sharvelle, and Hamilton (*8*) to control humidity, could be made in this apparatus, it was proved well suited to the purpose, which was to apply two or three different materials within a few minutes of each other.

Though Horsfall and his associates suggested as a standard surface that pyroxylin (cellulose nitrate) be dissolved in butyl acetate and deposited on glass, the present writer prepared the cellulose nitrate in the laboratory, and dissolved it in three parts of ether to one of alcohol. The surfaces were prepared by dipping microscope slides into this solution and standing the slides vertically in a dust-free atmosphere to dry for 24 hours.

Since the applicator was found to deliver bordeaux and bordeaux plus oil (hereafter called "oil-bordeaux" except where the type of oil is specified) at the same rate, some definite time or stage had to be established as an end-point in application. Evans and Martin (*2*) had applied the spray until the liquid began to run down the surface. Hoskins and Ben-Amotz (*9*), on the other hand, ended application after a measured amount of liquid had drained from the surface. In the present work certain considerations guided the decision to end application at the two

following stages: (1) at the point just before the liquid began to run down the surface (runoff stage), and (2) when about 1 cc of liquid had drained from the surface (drip stage). The considerations were as follows: assuming equal delivery of two materials from the atomizer, it is evident that at least during the early stages of application the liquid will be deposited at equal rates. If, therefore, two materials were applied for the same length of time, they should deposit the same amount, provided one material did not begin to run off the surface before the other. The runoff stage provides, therefore, a criterion to judge retention of the liquid by the surface, for if one liquid is retained less than another this liquid will require the shorter period of application to reach the runoff stage. Since in practice one cannot spray all parts of the tree to exactly the same stage as regards runoff, one should determine whether overspraying causes differences in deposits; slides were, therefore, sprayed until about 1 cc of liquid had drained from the surface (drip stage).

TABLE 8

EFFECT OF PETROLEUM-OIL EMULSION ON RETENTION OF BORDEAUX DEPOSIT BY A CELLULOSE NITRATE SURFACE

Material	Stage at which application was stopped	Milligrams of copper on 1 square centimeter of surface
Bordeaux, 1 per cent	Runoff*	0.0318
Bordeaux, 1 per cent, plus 3 per cent of emulsion A	Runoff*	0.0343
Bordeaux, 1 per cent	Drip†	0.0211
Bordeaux, 1 per cent, plus 3 per cent of emulsion A	Drip†	0.0252
Difference required for significance: 19:1 odds	.....	0.0019
Difference required for significance: 99:1 odds	.....	0.0026

* Spraying was stopped when liquid showed signs of beginning to run down the surface.

† Spraying was stopped when 1 cc (approximately) of liquid had accumulated at the lower end of the slide.

Table 8 gives a typical example of spraying 10 slides each with bordeaux 1 per cent (8–8–100, approximately) and bordeaux 1 per cent plus 3 per cent of petroleum-oil emulsion A to the runoff stage and to the drip stage. The deposit of oil-bordeaux is seen to be significantly higher than that of bordeaux without oil when spraying was carried to either stage. According to these data, furthermore, significantly higher amounts of copper were present on surfaces sprayed only to the runoff stage than on surfaces sprayed until drip occurred.

In five such tests, when application ended at the runoff stage, bordeaux

with 3 per cent of oil emulsion increased deposits by 37 per cent, whereas the increase required for statistical significance at 99:1 odds was 21 per cent. In similar tests, when application was continued to the drip stage, oil-bordeaux increased deposits 26 per cent, whereas the increase required for significance at 99:1 odds was 20 per cent.

As was said earlier, the sprayer delivered oil-bordeaux and bordeaux at the same rate. If we assume, therefore, that the two materials were deposited on the surfaces at the same rate, the amount of each present at the runoff stage should differ only if the period of application necessary for producing runoff was longer with one than with the other. To test this point, one lot of bordeaux was divided into three portions. To the first was added 2 per cent of oil emulsion A; to the second, ¼ per cent of an organic spreading agent; to the third, nothing. The amount of bordeaux in all lots was adjusted to the same value by adding the requisite amount of water. In applying these materials to cellulose nitrate-covered slides, the time necessary to reach the runoff stage and the drip stage, respectively, was determined with a stop watch. On ten slides the average time necessary to produce runoff with bordeaux was 5.6 seconds; with bordeaux plus oil emulsion A, 7.2 seconds; with bordeaux plus the organic spreading agent, 4.4 seconds. The time necessary to reach the drip stage was 13.0, 16.1, and 9.7 seconds, respectively. Apparently, therefore, the time factor is one cause of the greater deposit of oil-bordeaux.

It is noteworthy that bordeaux with the spreading agent required the shortest application periods. Hockenyos and Irwin (*6*) found a similar situation when applying bordeaux with certain supplements to peach leaves. The present results with the spreader seem significant, furthermore, in view of the evidence by Evans and Martin (*2*) and Hoskins and Ben-Amotz (*9*) that an increase in the wetting and spreading quality of a spray material was frequently accompanied by a lower deposit. The relation of wetting and spreading to distribution of the deposit was next studied.

EFFECT OF OIL ON THE DISTRIBUTION OF BORDEAUX OVER THE SURFACE

According to studies by Evans and Martin (*2*), an accessory material that promotes wetting and spreading of the aqueous phase increases the uniformity with which the fungicide suspended therein is distributed over the sprayed surface. On surfaces particularly difficult for water to wet, the fungicide is deposited in unevenly distributed patches. Thus Yarwood (*16*) found bordeaux to be deposited on onion so unevenly as to be ineffective against the downy mildew fungus. When, however, a

spreading agent (Penetrol) was added, the bordeaux was distributed more evenly over the leaves and, in consequence, controlled the mildew more effectively.

Since peach twigs did not furnish a satisfactory surface for studying the effects of oil on distribution of bordeaux, the following tests were conducted with cellulose nitrate on glass slides. Bordeaux and oil-bordeaux exhibited little difference in the distribution of deposit on this surface when application was stopped before the runoff started. If, however, application was continued until 1 or 2 cubic centimeters of liquid had drained from the surface, the resulting deposit of oil-bordeaux was more even and finer in grain. The reason for this difference was clearly seen in observing the manner in which the bordeaux and oil-bordeaux drained from the surface. Whereas the former flowed down the slides in a series of drops which followed irregular courses and, in consequence, left uneven deposits of the solid, the oil-bordeaux flowed down the surface as a sheet, leaving the solid more evenly distributed.

When, immediately after deposition by spraying, the individual droplets of bordeaux and oil-bordeaux were more closely studied, those of the latter were found to cover somewhat smaller areas. To examine this point further, bordeaux, with and without different combinations of oil and a wetting agent were dropped from a 1-cc pipette from a height of 1 inch upon horizontal surfaces of cellulose nitrate. After 15 minutes the diameters of the drops were measured. The volume of the drops delivered by the pipette was established for each material. Table 9 gives data on the spread of drops, the volume of drop, and a percentage relation between these two data which was obtained by dividing the diameter of the drop (resting on the surface) by its volume. The ratios thus obtained were then expressed as a percentage of the ratio for water; that is, the value for water was considered as 100.

According to table 9 the diameter of spread of 1 per cent bordeaux was 90 per cent of that of water, whereas the spread of bordeaux plus 2 per cent of oil emulsion A was 63 per cent, and bordeaux plus oil without the emulsifier was even less. The spread of oil emulsion A (used alone) was practically the same as that of bordeaux plus oil emulsion A.

The organic spreading agent more than doubled the spread of water, noticeably increased the spread of bordeaux, and overcame the tendency for oil to restrict the spread of bordeaux. Since the amount of organic spreading agent was much more than the amount of emulsifier introduced into the spray with oil emulsion A, this is not a fair comparison of the spreading efficiency of the two. The differences in the results secured serve, however, to emphasize the relatively small influence the

emulsifier in emulsion A had on the spreading of oil-bordeaux under conditions favorable to spreading.

The conditions of these tests favored spread[6] because the time interval elapsing between deposition and measurement of the drops allowed for possible extension of the liquid over the surface by capillarity. In this respect, among others, the conditions of the tests differ from those exist-

TABLE 9

Effect of Different Materials on the Area Covered by a Drop of Bordeaux Mixture on a Cellulose Nitrate Surface

Material*	Mean diameter of drops on surface, millimeters†	Volume of drops, cubic centimeters	Percentage relations between data‡
Water	10.1	0.051	100
Bordeaux	8.9	0.050	90
Bordeaux, plus 2 per cent of oil emulsion A	6.1	0.049	63
Bordeaux, plus 2 per cent of tank-mix oil (emulsified by the bordeaux)	5.8	0.050	59
Oil emulsion A, 2 per cent, in water	6.4	0.050	65
Bordeaux, plus 2 per cent of tank-mix oil plus ½ per cent of organic spreader	6.5	0.033	99
Bordeaux, 1 per cent, plus ½ per cent of organic spreader	7.8	0.038	104
Organic spreader, ½ per cent in water	12.6	0.027	236
Difference required for significance: 19:1 odds	0.7	.....	...
Difference required for significance: 99:1 odds	0.9	.....	...
Calculated F value	99.6	.....	...

* Bordeaux was prepared from 1 part of copper sulfate, 1 part of lime, and 100 parts of water, approximately 8–8–100.

† Measurements made 15 minutes after the material was deposited on the slide.

‡ The percentages were calculated as follows: By dividing the diameter of the drop on the surface by its volume, a ratio was obtained for each set of values. For water the ratio was considered 100 per cent; the others were referred to this basis.

ing under actual spray application. Where spray is applied, the liquid on the surface is constantly being disturbed by oncoming spray and, in consequence, the surface forces that determine its wetting and spreading properties do not attain equilibrium. The force of impact of spray with the surface is a second factor not of particular influence in these tests. Gravity in its tendency to pull the spray droplets down the vertical or inclined surface is a third factor that was not operative. Therefore, one cannot conclude that the same differences in the area covered by the individual droplets of bordeaux and oil-bordeaux will necessarily occur under actual spraying conditions. Some difference does exist, as was indicated by the observations, noted earlier, that the areas covered by

[6] For a discussion of the wetting and spreading properties of liquids, and the influence of time on the expression of these properties, see Evans and Martin (*2*), Hensill and Hoskins (*5*), and Hoskins and Ben-Amotz (*9*).

individual droplets of the two types of bordeaux deposited by the applicator were not the same, those of oil-bordeaux being smaller than those of bordeaux. Whether an increase in the pressure of application would modify these differences is not known. A greater force of impact of spray with surface might force the droplets of oil-bordeaux to flatten out and cover a greater area even though the liquid did not wet this area. Droplets of bordeaux would probably be affected likewise.

Although the effect of impact pressure on spreading of the spray droplets was not studied, the following tests indicate that within the interval between deposition and drying, neither bordeaux nor oil-bordeaux spreads beyond the area they occupied when they were deposited. Drops were placed on horizontal cellulose nitrate surfaces in the manner described earlier. Their diameters were measured upon deposition and again after most of the water had disappeared. Whereas neither bordeaux nor bordeaux containing 3 per cent of oil emulsion A spread during this interval, bordeaux containing ¼ per cent of the organic wetting agent spread appreciably. Thus it would seem that the difference between the area covered by a given volume of bordeaux and oil-bordeaux was not due to difference in their spreading properties, but was probably (in part at least) the result of differences in wetting properties. A second factor, viscosity, also might have been partially responsible, but this phase was not studied.

The behavior of the solid (bordeaux precipitate) and oil phases was also observed during the drying of films deposited by the applicator. Whereas the solid phase showed no tendency to rearrange itself during this process, the oil behaved as follows: In freshly deposited drops the oil was visible as small globules enmeshed in the bordeaux precipitate. They retained their shape until most of the water had evaporated, but then broke from the emulsified state and spread over and among the solid particles. That the oil also deposited as a film on the surface was shown by the fact that an oil coating was left on the slides after the bordeaux had been removed with a weak acid.

EFFECT OF OIL ON TOXICITY OF BORDEAUX TO FUNGUS SPORES

If the release of soluble copper from the bordeaux film is requisite to toxic action, anything altering this release will affect toxicity. Holland, Dunbar, and Gilligan (*7*) believed that attempts to increase bordeaux tenacity might impair its toxicity. Goldsworthy and Green (*4*) claimed that some accessory materials rendered the particles of certain copper fungicides impervious to external solubilizing agencies.

Observations presented in the previous section suggest that when an oil-bordeaux film dries, the oil coats the bordeaux precipitate. At the time spray is applied the oil is dispersed as droplets among the bordeaux particles, but upon disappearance of water from the film the oil droplets were seen to break and spread over and among the bordeaux particles. The question of whether this coating of oil affected toxicity of bordeaux to fungus spores was studied.

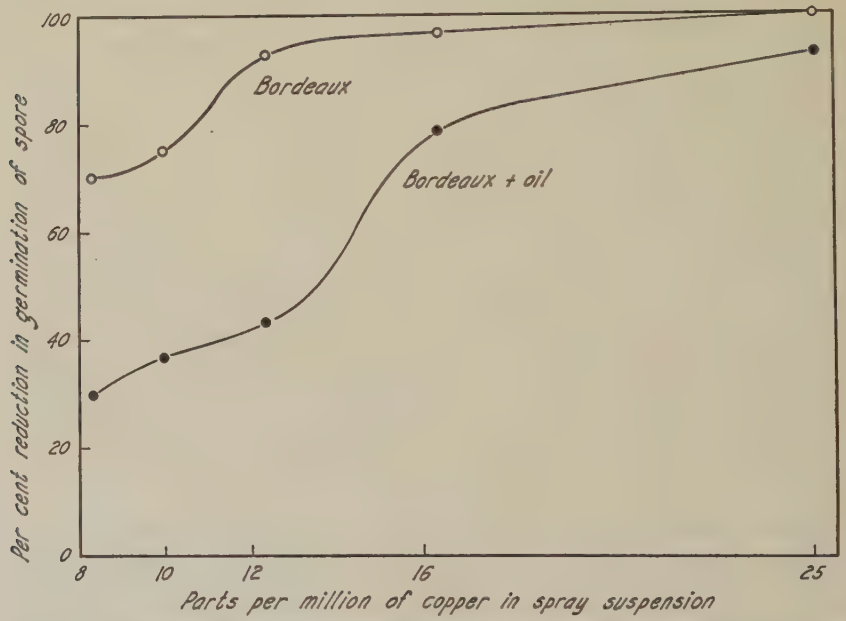

Fig. 1.—Effects of diluting bordeaux and bordeaux plus 3 per cent of oil on the toxicity of their dried films to germination of spores of *Coryneum Beijerinckii*. Undiluted (8–8–100) bordeaux of both types proved highly toxic, but with dilution oil-bordeaux was reduced in toxicity more rapidly than bordeaux.

As the tests showed, freshly prepared films of bordeaux (8–8–100) and of bordeaux (8–8–100) plus 3 per cent of oil have such uniformly high toxicity to spores as to make comparisons at this concentration impossible. Spores of neither *Coryneum Beijerinckii* nor *Sclerotinia fructicola* germinated when placed over the two types of dried films. The two types of bordeaux, therefore, were diluted successively; samples of each dilution were dried on glass slides; and spores of the two fungi suspended in sterile, distilled water were placed over the dried films. Figure 1 represents the percentages of reduction in germination of the spores of *Coryneum Beijerinckii,* plotted against parts per million of copper in the bordeaux suspension. Bordeaux was consistently more

effective in reducing germination than oil-bordeaux, both with *Coryneum Beijerinckii* and with *Sclerotinia fructicola.*

A second type of experiment was performed as follows: Bordeaux and bordeaux plus 2 and 4 per cent of oil emulsion A were diluted to contain 1 part of copper in 22,000 parts of water. Samples of these were deposited and dried on glass slides. An elongated drop of water, containing about 25 spores per low-power microscopic field, was so placed that one end

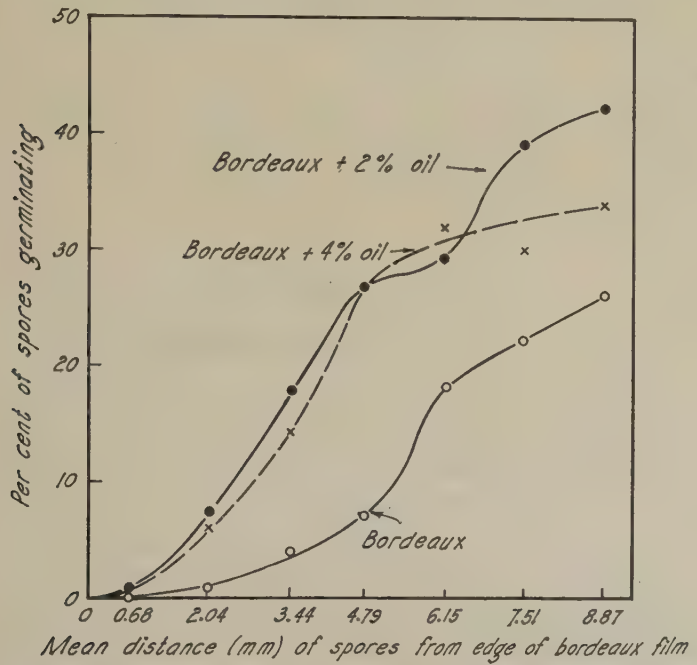

Fig. 2.—Effect of 2 and 4 per cent of oil emulsion on the toxicity of a dried deposit of bordeaux (8–8–100, diluted to contain 1 part of copper in 22,000 parts of water) to spores of *Coryneum Beijerinckii* lying in water at different distances from the deposit. Bordeaux suppressed germination more than oil-bordeaux at all distances from the deposit.

covered the bordeaux film, the other extending over clean glass for a distance of about 10 millimeters. After 24 hours the percentage of spores germinating was determined in zones located at different distances from the edge of the bordeaux film. According to the data in figure 2, no germination occurred when the spores were located over the film; but as the distance from the film increased, so did germination. The curve for germination over oil-bordeaux films rises more rapidly than that for germination over bordeaux films, indicating, as did the first type of experiment, a somewhat lower toxicity of oil-bordeaux.

These results represent the level of toxicity of freshly deposited bordeaux films only. That weathering alters the composition of a bordeaux film was recently demonstrated by Wilcoxon and McCallan (*11*), who found that the first change undergone—the carbonation of the excess lime—was completed within a few hours. The next change, brought about by rain, was the preferential removal of calcium and sulfate. When considerable amounts of these are removed the film becomes proportionately richer in copper. In freshly prepared bordeaux films, copper was only slightly soluble; but as leaching by rain continued, the solubility became greater.

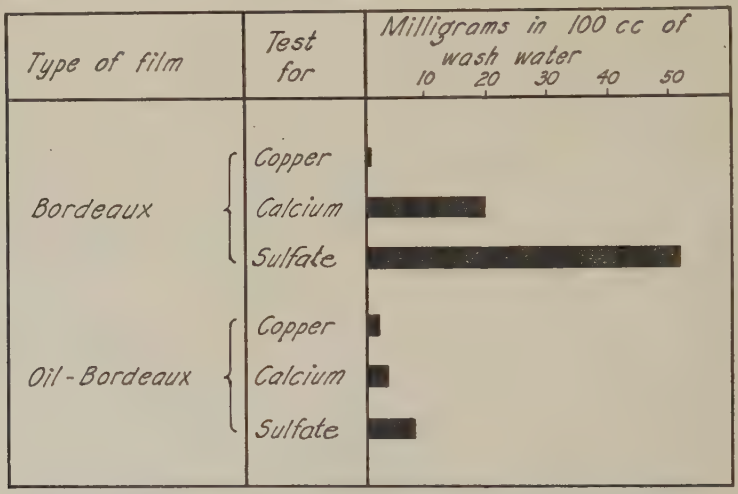

Fig. 3.—Effect of 3 per cent of oil emulsion on the amount of soluble copper, calcium, and sulfate leached from a bordeaux deposit with distilled water applied as "rain." Much of the copper lost from the oil-bordeaux deposit was dissolved in oil that escaped during leaching.

The effect of oil on leaching was therefore studied. Bordeaux (8–8–100) and bordeaux (8–8–100) plus 3 per cent of oil emulsion were deposited in equal quantities in uniform petri dishes. After drying for several days the plates were placed at a slight angle in separate funnels equipped with filter paper. Distilled water was atomized over the surfaces of the films for definite periods. The wash water was collected and analyzed for calcium, sulfates, and copper. In all cases oil retarded the loss of calcium and sulfates, but increased the loss of soluble copper from the films (fig. 3).

A slight turbidity of the wash water from oil-bordeaux film indicated that some material escaping from the film was capable of passing through filter paper. This material proved to be oil, which, upon extraction by

petroleum ether, proved to contain copper. In fact, most of the copper in the wash water was held by the oil. That the oil dissolves copper from the bordeaux residue was further shown by extracting oil-bordeaux film with anhydrous ether, after thoroughly drying the film in warm air for several hours. Upon evaporating the ether, incinerating the oil, and dissolving the residue in nitric acid, copper was found in considerable amounts.

Though the amounts of copper in wash water from the two types of films was not compared after long-continued leaching, the wash water from oil-bordeaux contained more copper than that from bordeaux at the end of the fourth 1-hour leaching. Obviously, as long as the oil is being removed from the film, the copper dissolved therein will also escape.

Whether or not the oil-dissolved copper renders the wash water more toxic to fungus spores than water from bordeaux films was the next problem. The following are results of a typical experiment: Spores of *Sclerotinia fructicola* suspended in water which had leached a bordeaux film for 20 minutes and into which had escaped 0.2 p.p.m. of soluble copper, and suspended in water which had leached an oil-bordeaux film for 20 minutes and into which had escaped 0.9 p.p.m. of soluble copper (most of which was dissolved in oil), germinated 73 and 61 per cent respectively, as compared with 89 per cent for the check. Other experiments with spores of *Coryneum Beijerinckii* indicated still smaller differences between the toxicities of wash waters from the two types of films. Under conditions of these experiments, therefore, the results were such as to suggest that the oil-dissolved copper escaping during leaching imparts little or no additional toxicity to the water. The results of toxicity studies presented in figures 1 and 2 are in accord with this conclusion, inasmuch as unleached oil-bordeaux was slightly less toxic than bordeaux.

DISCUSSION

The results secured in different phases of this study can now be related to one another. Attention is first directed to the evidence regarding loss of bordeaux from the sprayed surfaces. Wilcoxon and McCallan (*11*) described the changes that meteorological conditions effect in a bordeaux film after deposition. First, the excess lime is carbonated by action of carbon dioxide; second, rain leaches calcium and sulfates from the film; and third, the copper increases in solubility and is presumably washed away by rain. Just how rapidly copper is lost from the film by this leaching process is not known. It is probably slower than the loss that results when the bordeaux precipitate, as such, is removed from the surface by the eroding effect of rain. Since the field evidence secured

in this study does not distinguish between these two types of loss, the role played by oil in preventing loss of both types is not revealed. In laboratory "weathering" tests, however, the oil decreased the loss of calcium and sulfate by leaching. If, as indicated by Wilcoxon and McCallan's studies (*11*) water-soluble copper increased measurably only after disappearance of considerable calcium and sulfate from the film, then oil probably also delays leaching of copper. As will be remembered, the greater part of soluble copper escaping from oil-bordeaux film during laboratory weathering tests was dissolved in the oil; and the amount lost, in consequence, was limited to the dissolving power of the oil. The oil probably did not increase the amount of water-soluble copper, but very likely decreased it by protecting the bordeaux particles against the leaching effect of rain.

That oil protects the bordeaux from external weathering agencies might also be inferred from the observations that upon drying of an oil-bordeaux film the oil droplets broke from their emulsified state and spread over and among the bordeaux particles. The degree to which the bordeaux is oil-coated depends, of course, on the amount of oil used. In this connection one should remember that the tenacity of bordeaux increased with increasing amounts of oil. During the early stages of weathering the increased tenacity may very likely be due to a water-repellent property conferred upon the bordeaux and the surface by the oil. According to views expressed by Fajan and Martin (*3*), certain surface-active substances may reduce the tenacity of fungicides because they render the fungicide deposit more wettable by rain. Rendering the fungicide deposit less wettable by the addition of oil may therefore increase tenacity.

As certain emulsifying agents are good wetting agents, their tendency to increase the wetting properties of bordeaux film after it is deposited and dries should not be disregarded. In the present studies the emulsifier present in oil emulsion A was not shown to influence the wettability of the surface by the liquid oil-bordeaux. The reason for this might be that the emulsifier was not an active wetting agent; but a more likely explanation is that the emulsifier was present in the spray in such small amounts. When comparatively large quantities of an organic wetting agent were added to bordeaux, the wetting properties of the spray were increased. Possibly, therefore, an emulsifier that has high wetting powers and is used in amounts greater than in these tests might modify the tendency for oil to increase the tenacity of bordeaux.

Inasmuch as the evidence regarding the effect of oil on both the amount and the distribution of bordeaux deposit is intimately connected with

the wetting and spreading properties of the liquid, these phenomena must be considered. The wetting property of a liquid is that property which enables the liquid to make stable contact with the surface; the spreading property, on the other hand, determines the extent to which the liquid spreads over the surface by capillary forces (*2, 5, 9, 15*). The former determines the extent and the persistency of the contact of liquid with surface, and probably depends somewhat on time for maximum expression; the latter determines the final area covered by a given volume of liquid, and definitely depends on time for its maximum expression. In the present studies, the observation that upon hitting the surface the spray droplet did not run off, but occupied a certain area and assumed a definite shape, illustrated the degree to which the liquid wet the surface. If the surface proved nonwettable, the droplets would roll off it without leaving a liquid deposit behind. Drops of bordeaux and oil-bordeaux placed upon a horizontal cellulose nitrate surface did not increase the area they occupied upon hitting the surface—an observation that illustrates the low capillary activity of the sprays on the particular surface. According to these tests, therefore, the addition of oil to bordeaux decreased the wettability of the latter, but did not affect the spreading properties. Hoskins and Ben-Amotz (*9*) found that an oil-water emulsion containing blood albumin or hemoglobin as the emulsifier would wet beeswax surfaces less easily than the corresponding water solutions of blood albumin or hemoglobin.

When bordeaux and oil-bordeaux were applied to a cellulose nitrate surface, the latter formed drops that occupied less area than those of the former. To cover a given area completely, therefore, a greater number of oil-bordeaux drops must be applied. Assuming that the time in application when the spray began to run from the surface was the point at which the surface was completely covered by liquid, then at this time the film of oil-bordeaux would be thicker than the film of bordeaux, because it requires longer application to produce runoff. Although this view fits the evidence secured, oil-bordeaux may have required a longer period of application than bordeaux to reach the runoff stage because it was more viscous and resisted the tendency of gravity to pull it from the position it occupied upon deposition (*15*).

From the beginning of application until the runoff stage the amount of deposit increased. When the liquid began to drain from the surface, however, no further increase could occur; on the contrary, the deposit of bordeaux decreased 33 per cent, and that of oil-bordeaux, 27 per cent. It was during the runoff stage that oil was found to improve the distribution of the bordeaux precipitate over the surface.

Though laboratory tests indicated oil-bordeaux to be slightly less toxic to fungus spores than bordeaux, the field tests revealed no such difference. The former, in fact, gave better control because it remained on twigs over a longer period. Under ordinary conditions, therefore, the slight effect of difference in toxicity is likely to be nullified by the greater effect of increased tenacity.

SUMMARY AND CONCLUSIONS

For adequate protection of peach trees against the attack of *Coryneum Beijerinckii* the twigs must be protected by a fungicide throughout the winter. The weather-resisting quality (tenacity) of the fungicide is therefore a determining factor in successful control. The primary purpose of field tests reported herein was to determine how certain added materials, particularly petroleum-oil emulsion, affect the tenacity of bordeaux mixture. The influence of oil on retention, coverage, and toxicity of bordeaux was studied in the laboratory.

When used in sufficient amounts, a dormant-type petroleum oil increased tenacity of bordeaux mixture. Thus 3 or 4 per cent decreased the loss of copper from peach twigs during the winter, but 1 per cent did not. This was true both of a commercial emulsion and of a tank-mix oil emulsion made with blood albumin.

By preventing loss of copper, the oil prolonged the period of protection afforded by a single treatment of bordeaux given in the autumn. In one year when *Coryneum Beijerinckii* attacked the twigs throughout the winter, the greater protective efficiency of oil-bordeaux was reflected in better control. In another year, however, when attacks of the fungus were confined to early winter, before rains had reduced copper deposits, bordeaux controlled the disease about as effectively as oil-bordeaux.

Neither bentonite (0.6 and 2 pounds per 100 gallons of spray) nor cottonseed oil (1 per cent) appeared to affect the tenacity of bordeaux or its control of twig infection by *Coryneum Beijerinckii*.

None of the added materials affected the efficiency with which bordeaux controlled leaf curl (caused by *Taphrina deformans*). A single autumn application of bordeaux, with or without added materials, effectively reduced the disease.

In certain field tests, oil appeared to affect the amount of bordeaux retained by peach twigs; but the results varied in such a way as to be inconclusive. Laboratory tests, therefore, were performed to determine the retention of bordeaux and oil-bordeaux by vertical surfaces of cellulose nitrate. The sprays were applied with an atomizing apparatus designed to deliver a constant volume of liquid.

When bordeaux and bordeaux plus 3 per cent of oil emulsion A were applied until the liquid showed signs of running down the surface, the latter deposited an average of 37 per cent more copper than the former. When application was prolonged until approximately 1 cc of liquid had run off the surface (drip stage), the latter deposited 26 per cent more copper than the former. With both types of bordeaux the deposit was greater at the runoff stage than at the drip stage. Between these two stages the copper deposit decreased 33 per cent for bordeaux, 27 per cent for oil-bordeaux.

In trials where the length of time necessary to produce runoff of the liquid from the slide was determined, oil-bordeaux was found to require the longer period.

The effect of petroleum-oil emulsion on coverage, or distribution of bordeaux over the cellulose nitrate surfaces, was observed by examining the area of surface covered by droplets sprayed onto slides. The area covered by a droplet of oil-bordeaux was found to be smaller than that covered by a droplet of bordeaux, as was also the case when equal-sized drops of the two types of bordeaux emitted from a pipette were allowed to fall from a fixed distance onto horizontal cellulose nitrate surfaces. As these drops neither extended nor contracted after deposition and before drying, spreading by capillary forces was apparently not present to any extent. Therefore, upon coming in contact with a surface during application, the area covered by the spray droplets was largely determined by the wetting properties of the liquid. As bordeaux wetted the surface somewhat better than oil-bordeaux, the droplets of this material occupied the larger area; and as fewer were required to cover the surface, less bordeaux than oil-bordeaux was required to produce runoff; hence less was retained by the surface. Difference in viscosity of bordeaux and oil-bordeaux might account, in part, for differences in deposits, although this phase was not studied. Application of the spray at a pressure higher than that employed in these tests might modify this difference in behavior between the two types of bordeaux. Force of impact would force the droplets to spread over areas they do not wet, although they might tend to withdraw from these areas after application ceases.

When application was prolonged until liquid had drained from the surface and the retained precipitate was dried, the oil-bordeaux was found more evenly distributed than the bordeaux. There is, apparently, an explanation: whereas bordeaux drained from the slide in a series of large drops which pursued an uneven course down the slide, leaving heavy and light deposits in their wake, oil-bordeaux drained from the surface as a sheet, leaving a more even deposit.

Though the laboratory results thus far are not adequate to explain the different problems encountered in the field, they contain suggestions that would account for wide variabilities in deposits between years, or between applications made by different individuals. For example, a tendency to end application at the time the spray begins to drip from the trees might give certain deposits, whereas a tendency to overspray might give different deposits.

The toxicities of the two types of bordeaux to spores of *Coryneum Beijerinckii* and *Sclerotinia fructicola* were compared in laboratory studies. Bordeaux mixtures (8–8–100), with and without oil, were of such uniformly high toxicity as to be indistinguishable in this regard. When, however, successive dilutions were made, bordeaux appeared to be somewhat more toxic than oil-bordeaux. Another method of assessing toxicity gave similar results. This method consisted in germinating spores of the two fungi in elongated drops of water, which were placed on slides with one end of the drop resting over a dried film of the fungicide, the other end extending for several millimeters over clean glass. The percentages of spores germinating were determined in zones at different distances from the edge of the fungicide deposit and were greater with oil-bordeaux than with bordeaux.

In artificial weathering tests, 2 per cent or more of oil emulsion was found to reduce the loss of calcium and sulfate from dried bordeaux films. As will be recalled, Wilcoxon and McCallan (*11*) found that during weathering, a loss of these two constituents was the forerunner of an increase in soluble copper in dried bordeaux films.

In tests of wash water from weathered bordeaux and oil-bordeaux films, more soluble copper was found to escape from the latter than from the former. It was determined, however, that most of the soluble copper was held by oil which escaped during the weathering process. Oil extracted from thoroughly dried oil-bordeaux films by anhydrous ether contained considerable amounts of soluble copper. The presence of this oil-held soluble copper did not appear to increase markedly the toxicity of the wash water to fungus spores.

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PHYTOPHTHORA CINNAMOMI AND WET SOIL IN RELATION TO THE DYING-BACK OF AVOCADO TREES

VINCENT A. WAGER

PHYTOPHTHORA CINNAMOMI AND WET SOIL IN RELATION TO THE DYING-BACK OF AVOCADO TREES[1,2]

VINCENT A. WAGER[3]

INTRODUCTION

A DYING-BACK or decline of avocado trees has become a serious problem to growers in some parts of southern California during the last few years.[4] The trees affected are usually those that are fairly old (ten or more years of age), and the trouble may occur in isolated trees or, more commonly, in groups of trees in an orchard.

Horne (*7*)[5] describes this decline under the various names of melanorhiza, water injury, asphyxiation, apoplexy, and collapse, and associates it with such conditions as excess water, lack of aeration, and heavy subsoils, not with any particular organisms.

Affected trees appear to lose vitality; they become sparsely foliated, fail to produce crops, and their branches begin to die back. Such trees have been seen occasionally growing in sandy soil where drainage conditions would appear to be good. But in many instances, when holes were dug alongside of these trees, an impervious subsoil was found about 2 feet below the surface.

The possibility that at times the decline of the trees is caused by too much water, cannot be overlooked. In one instance, a hole approximately 3 feet deep was dug in an affected orchard some 10 days after a period of continuous, fairly heavy rain in midwinter. In about 15 minutes, water began to ooze out of the sides of the hole, at a depth of about 2 feet from the surface of the ground, and to trickle to the bottom.

Roots of most of the trees examined were found to be blackened and dead, especially the fibrous roots and those up to ⅛ inch in diameter. Larger roots, ¼ to ½ inch in diameter, also, were sometimes soft, brown,

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[2] Paper No. 455, University of California Citrus Experiment Station, Riverside, California.
[3] Plant Pathologist, Union of South Africa Department of Agriculture. On Commonwealth Fellowship in collaboration with the Division of Plant Pathology, University of California Citrus Experiment Station, Riverside, California, September, 1939, to June, 1940.
[4] Mr. M. B. Rounds places the number of acres of avocados affected with this trouble, conservatively, at 500.
[5] Italic numbers in parentheses refer to "Literature Cited" at the end of this paper.

and rotten and had a disagreeable odor. When the thin bark of the large roots was scraped with a knife, brown lesions ¼ to ¾ inch in size were often seen, usually at the junction of a small root which was dead. When the thin bark of healthy roots is scraped, the underlying tissue is found to be white and crisp; in affected roots this tissue is brown and soft.

A few large trees have been known to die suddenly. One such tree was a twenty-five-year-old avocado with a trunk nearly 18 inches in diameter. All the leaves of this tree withered and died suddenly during the month of September, 1939, after a period of particularly hot weather, and many of its roots were found to be blackened and dead. The theory was advanced that the tree, which was standing in a slight depression, had, during the winter, received too much water; that many roots had consequently become infected with fungi and had died; and that, possibly, the sudden heat had caused excessive transpiration, with which the reduced root system could not cope.

FUNGI FOUND ON ROOTS OF AVOCADO

Cultures were made from 156 roots from affected avocado trees growing in seven different localities. In each case, cultures were made from fibrous roots, from small roots ⅛ inch in diameter, and from larger ones ¼ to ½ inch in diameter; cultures were also made from lesions on the big roots. A *Phytophthora* species was found on 37 roots from six of the seven localities, *Pythium* species were found on 21 roots from all seven localities, and *Fusarium* species on most of the remaining 98 roots from all localities. The *Phytophthora* species was found generally in the lesions and on the larger-sized roots—very seldom on the fibrous roots. The *Pythium* species were usually on the fibrous and smaller-sized roots.

The *Phytophthora* species found in the cultures was identified as *Phytophthora Cinnamomi* Rands. The *Pythium* species were identified as *Pythium vexans* de Bary (on 20 roots) and *Pythium ultimum* Trow (on 1 root). Two other fungi which very frequently appeared in these cultures were *Fusarium oxysporum* Schl. and *Cylindrocarpon radicicola* Wr.[6]

PHYTOPHTHORA CINNAMOMI RANDS

This is the first record of *Phytophthora Cinnamomi* on avocados in the United States. All the cultures obtained were found to be similar. The fungus grows well on various culture media and produces a tough, wiry, aerial mycelium.

Hyphae are as much as 8 μ in diameter and covered with irregularly shaped protrusions, with numerous septations in the hyphae in older

[6] These two fungi were identified by W. C. Snyder, Assistant Professor of Plant Pathology and Assistant Plant Pathologist in the Experiment Station.

cultures. Chlamydospores occur in bunches on short stalks and are usually spherical and thin-walled. In diameter, they range from 18 to 48 μ, are commonly 42, and average 37.8 μ. Oögonia are spherical and terminal; they range from 27 to 48 μ and average 37 μ in diameter. In color, they are golden brown. The oöspore practically fills the oögonium and is spherical and thick-walled. Antheridia are rounded, about 12 μ in diameter, and amphigynous. Sporangia are thin-walled, nonpapillate, and produced on long, thin hyphae; they vary from 30 to 80 $\times$ 20 to 45 μ in size. In one batch of material they were commonly 40 $\times$ 25 μ; in another, 60 $\times$ 40 μ; in a third, 75 $\times$ 45 μ. The sporangial stalk may continue to grow through and out of an empty sporangium and produce another; or, more commonly, new sporangia may develop within the old, empty sporangium. Sporangia were produced in abundance when the fungus, grown on sterilized wheat, was placed in running water. As many as 16 zoöspores were seen in a sporangium. They are actively motile on liberation and soon round off to a diameter of 10 μ.

Rands (*10*) in his description of this fungus gives measurements of chlamydospores as 28 to 60 μ, average 41 μ; sporangia, 25 to 100 $\times$ 18 to 43 μ, average 57 $\times$ 33 μ; oöspores were not observed. Ashby (*1*) found that oögonia averaged 32 μ in diameter. Tucker (*11*) obtained oögonia 28 μ in diameter. Thus, except for larger oögonia, the description of the fungus found in the avocado cultures is very similar to other descriptions of *Phytophthora Cinnamomi*. The oögonia obtained in the present study were from 3-month-old cultures in oatmeal tubes, which had been standing in the laboratory during the winter months.

Phytophthora Cinnamomi had previously been obtained from the roots of avocado trees suffering from dieback in South Africa (*14*). The fungus was very similar to that described above: the chlamydospores ranged from 26 to 43 μ and were commonly 32 μ in diameter; oögonia were from 30 to 52 μ in diameter and averaged 41.4 μ; sporangia were 39 to 66 $\times$ 26 to 40 μ, commonly 50 $\times$ 32 μ.

There appears to be considerable confusion in literature with regard to the taxonomy of *Phytophthora Cinnamomi* and *P. cambivora* (Petri) Buis. The latter is responsible for the "ink disease" of chestnuts in Europe. White (*16*) and Mehrlich (*8*) agree that the two fungi are but strains of the same species and hence retain the prior name of *P. cambivora*. Tucker,[7] however, states that he is inclined to agree with Ashby (*1*) in his retention of the two species, and this view has been adopted in the present study.

Phytophthora Cinnamomi has been recorded as follows: on cinnamon

[7] Tucker, C. M., in letter to author dated April 27, 1940.

(*Cinnamomum Burmanni* Bl.) in Sumatra (*10*); on chestnut (*Castanea sativa* Mill.) in England (*4*); on avocado (*Persea americana* Mill.) in Puerto Rico (*11*) and in South Africa (*13*); on American chestnut (*Castanea dentata* [Marsh] Borkh.), hairy chestnut (*C. mollissima* Blume), Japanese chestnut (*C. crenata* Blume), Japanese yew (*Taxus cuspidata* Sieb. and Zucc.), Norway spruce (*Picea Abies* [L.] Karst.), red pine (*Pinus resinosa* Ait.), Scotch pine (*P. sylvestris* L.), Colorado spruce (*Picea pungens* Engelm.), black walnut (*Juglans nigra* L.), Persian walnut (*J. regia* L.), birch (*Betula papyrifera* Marsh), oak (*Quercus borealis* Michx., *Q. montana* Willd., *Q. alba* L.), plane (*Platanus orientalis* L.), and locust (*Robinia Pseudo-Acacia* L.) in the southeastern United States (*3*); on rhododendrons (*Rhododendron californicum* Hook., *R. carolinianum* Rehd., *R. ponticum* L.) in the United States (*16*); on walnut in Australia; on heath (*Erica* sp.) in the United States and (*Erica hyemalis* Nichols, *E. nivalis* Andr., × *E. Willmorei* Knowles and Weste.) in England (*9*); and on sour orange (*Citrus Aurantium* L.) infected with gummosis in Brazil (*5*). *P. Cinnamomi* has also been reported in connection with wilt produced by inoculation in *Antirrhinum, Calceolaria, Schizanthus,* and beech (*Fagus* sp.) seedlings in England (*9*); and as causing a rot of pineapples (*Ananas sativus* Schult.) in Hawaii, Queensland, Costa Rica, Jamaica, Cuba, Haiti, and the Philippines (*9*). The fungus was recently isolated by W. T. Horne from lesions or cankers on the trunk of a Nabal avocado tree that was dying in San Diego County, California.

Temperature Requirements.—This fungus makes good growth over a fairly wide range of temperatures. The strain from South Africa grew well at temperatures ranging from 16° to 31° C, while that from citrus in Brazil made good growth also at 34°, the optimum for the former being 25° and for the latter, between 28° and 31° (*15*). The temperature requirements for the California fungus were not determined, but they are probably somewhat similar to those given above.

Soil Acidity in Relation to Phytophthora Cinnamomi.—White (*16*), in a study of rhododendron wilt caused by *Phytophthora Cinnamomi,* found that 60 to 100 per cent infection took place in infected soils ranging in pH value from 4.0 to 7.3. In a plot having a pH value below 4.0, only 1 plant out of 15 wilted; and in plots having a pH value above 7.3, there was 33 per cent mortality.

According to Haas (*6*), avocado seedlings grow better both in culture solutions and in soils having low pH values, the lowest tested being pH 4.5.

PYTHIUM SPECIES

Pythium vexans de Bary has not been previously recorded from avocado. This fungus was isolated from 20 different avocado roots from seven localities in southern California. It grows well on most culture media and has a distinctive type of growth suggestive of combed silk. The hyphal main branches are as much as 6 μ in diameter; side branches are thin (2 μ), and the tips are curly. Oögonia are spherical and terminal, usually on short side branches; commonly 18 to 22 μ, they average 20 μ in diameter. Antheridia have a large surface in contact with the oögonia and are usually funnel-shaped; they usually have a fairly long branch and may arise from a hypha not directly connected to the oögonium. Sporangia are generally spherical and terminal on short stalks (occasionally intercalar) 15 to 27 μ, mostly 21 μ, in diameter. Zoöspores are produced very readily, the evacuation tube being usually one half to one third as long as the diameter of the sporangium. Zoöspores were 7 to 8 μ in size, and the number produced in various sporangia ranged from 7 to 12.

This description agrees very closely with that of de Bary and with that of Braun (*2*) for the fungus he described as *Pythium complectens* Braun, which name, according to Middleton,[8] should be discarded in favor of *P. vexans.*

Pythium vexans was found in South Africa on papaya, or pawpaw (*Carica papaya* L.) infected with foot rot, and on perennial statice, or thrift (*Armeria* sp.), infected with wilt or crown rot (*14*). Middleton (see footnote 8) lists this fungus from the following hosts: alfalfa (*Medicago sativa* L.), sugar cane (*Saccharum officinarum* L.), durian (*Durio zibethinus* L.), pan (*Piper betle* L.), pipri (*P. longum* L.), stock (*Mathiola incana* [L.] R. Br.), castor bean (*Ricinus communis* L.), geranium (*Pelargonium* sp.), coleus (*Coleus* sp.), flax (*Linum usitatissimum* L.), rocket larkspur (*Delphinium Ajacis* L.), ginger (*Zingiber officinale* Roscoe), rubber (*Hevea brasiliensis* Muell.), carnation (*Dianthus Caryophyllus* L.), potato (*Solanum tuberosum* L.), and spinach (*Spinacea oleracea* L.). It was recently isolated on several occasions from the fibrous roots of citrus trees suffering from decline in California (*15*).

Pythium ultimum Trow was isolated from 1 root only. This is the first record of its occurrence on avocados. The fungus was typical of

[8] Middleton, John T. Taxonomy of the genus *Pythium* Pringsheim. Thesis in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of Missouri, 1940. (Typewritten.) Copy on file in the Library of the University of Missouri, Columbia.

Fig. 1.—*A, Phytophthora Cinnamomi* was added to the soil of each of these pots; a month later the 3 pots on the left were submerged for 2 days, the other 3 for 3 days. The pots were photographed 1 week later; by this time the avocado plants had wilted and died. *B,* No fungus was added to the soil in these pots; the 3 pots on the left were submerged for 3 days, the other 3 for 9 days. None of the plants subsequently showed any ill effects.

the species. *Pythium ultimum* is found commonly on citrus (*15*), but as it apparently plays little or no part in this avocado trouble, it is not discussed further in the present paper.

INOCULATION EXPERIMENTS

Previous inoculation experiments by the writer, in Africa, had indicated that under normal soil conditions *Phytophthora Cinnamomi* does not affect avocado plants adversely. Tucker (see footnote 7, p. 521), also, states that avocado plants growing under healthy conditions were not

Fig. 2.—*Phytophthora Cinnamomi* was added to the soil in the 4 pots on the left. Controls, on the right, were untreated. Soil in all pots was watered when necessary. Six months later all avocado plants were still healthy.

affected when the fungus was added to the soil; but if the pots were allowed to stand in saucers containing 1 inch of water, the plants rapidly wilted, while uninoculated controls treated in the same manner remained healthy. A series of experiments was therefore planned with the idea of simulating possible field conditions, where, as a result of continuous heavy rains or faulty irrigation practice, the soil becomes flooded and waterlogged for a short period of time. Tests were primarily with *Phytophthora Cinnamomi,* but a few tests with *Pythium vexans* were included in experiments 1 and 2.

Experiments 1 and 2.—For these experiments, trees two to three years old, growing in pots (figs. 1 and 2), were used. The soil in some pots was inoculated with *Phytophthora Cinnamomi,* that in other pots was inoculated with *Pythium vexans,* and that in the control pots was untreated. The fungi were grown in tubes of sterilized wheat kernels and

TABLE 1

Effect on Potted Avocado Plants of Inoculation of Soil and Submersion of Roots in Water for Various Periods of Time

Experiment no., inoculation treatment, and test no.	Plants	Time submerged	Results
	number	*days*	
Experiment 1:			
No fungus added to soil (controls):			
Test 1	4	0	Remained healthy; roots normal
Test 2	4	1	Remained healthy; roots normal
Test 3	4	3	Remained healthy; roots normal
Phytophthora Cinnamomi added to soil:			
Test 1	4	0	Three months later, all plants healthy; a few roots black and dead
Test 2	4	1	After 1 week, 2 plants slightly wilted (showed complete recovery after 2 months); 2 plants severely wilted and showing large number of roots blackened
Test 3	4	3	After 1 week, 2 plants dead, 2 severely wilted; roots mostly black and dead; lesions on main taproots; fungus recovered from most roots
Pythium vexans added to soil:			
Test 1	4	0	Remained healthy
Test 2	4	3	Remained healthy, except 1 plant which showed slight wilt after 1 week but recovered
Experiment 2:			
No fungus added to soil (controls):			
Test 1	4	3	Remained healthy; roots normal
Test 2	4	6	Remained healthy; roots normal
Test 3	4	9	Remained healthy, roots normal, except 1 plant which wilted and died 1 week later (*Pythium ultimum* isolated from blackened roots of this plant)
Phytophthora Cinnamomi added to soil:			
Test 1	6	2	Plants wilted after submersion; 2 plants recovered; 4 plants dead 1 week later; *Phytophthora Cinnamomi* recovered from most dead roots
Test 2	6	3	Plants wilted after submersion; all dead 1 week later
Pythium vexans added to soil:			
Test 1	4	2	Remained healthy
Test 2	4	3	Remained healthy

then introduced into a shallow hole in the top layer of the soil in the pots without injuring any roots. A month was allowed for the fungus to grow throughout the soil. Pots of each series were then immersed in larger containers of water for varying periods of time, after which the pots were lifted out of the water and allowed to drain rapidly. Results are presented in table 1.

TABLE 2

EFFECT OF INOCULATION WITH *Phytophthora Cinnamomi* ON AVOCADO PLANTS GROWN IN SOLUTION IN FLASKS

Experiment no., inoculation treatment, and test no.	Plants	Treatment of nutrient solution	Results
Experiment 3 (in laboratory; temperature about 23.9° C [75° F]):	*number*		
No treatment (controls):			
Test 1	3	Aerated	All plants remained healthy, but those aerated were more robust and developed more new roots than those not aerated
Test 2	3	Nonaerated	
Phytophthora Cinnamomi:			
Test 1	3	Aerated	After 1 week, all plants showed wilt and brown roots; after 2 weeks, all plants were dead
Test 2	3	Nonaerated	
Experiment 4 (in greenhouse; temperature 32.2° to 37.8° C [90° to 100° F]):			
No treatment (controls):			
Test 1	3	Aerated	All plants remained healthy, but those aerated had grown and produced more roots than those not aerated
Test 2	3	Nonaerated	
Phytophthora Cinnamomi:			
Test 1	3	Aerated	Wilted more slowly than similarly treated plants of experiment 3; dead after 3 weeks
Test 2	3	Nonaerated	

Experiment 3.—The soil was washed carefully from the roots of 6 avocado plants about 1 foot high. Sterilized wheat on which *Phytophthora Cinnamomi* was growing was scattered on the roots of these plants, which were then wrapped in damp paper for 2 days. Each plant was then placed in a 3-liter flask containing nutrient solution. Air was bubbled through the solution in 3 flasks, but not through that in the other 3.

Six other plants were used as controls. These plants were given the same treatment as that described in the preceding paragraph, except that there was no fungus on the wheat which was scattered on the roots.

All plants were kept in the laboratory, where temperatures reached about 23.9° C (75° F) and there was little air movement, so that transpiration was low.

Results of these tests are presented in table 2.

Experiment 4.—The soil was washed carefully from the roots of 12 avocado plants about 1 foot high. Each plant was placed in a 1-liter flask containing nutrient solution, and air was bubbled through the solution in all the flasks for 1 week.

Phytophthora Cinnamomi grown on sterilized alfalfa stalks and stimulated to produce sporangia and zoöspores freely by placing in running water,[9] was inserted in the neck of each of 6 flasks; sterilized stalks without the fungus were placed in the other 6 flasks, which served as controls. Air was bubbled through the solution in 3 of the flasks in each series, but not through that in the other 3.

Fig. 3.—Avocado plants in flasks containing nutrient solution through which air was bubbled continuously. *Phytophthora Cinnamomi* was placed in contact with the roots of the 3 plants on the right. The photograph was taken 10 days later, by which time these 3 plants had wilted and died. In a second similar series, not aerated, the results were indistinguishable from those shown here.

All plants were kept in the greenhouse, where temperatures reached 32.2° to 37.8° C (90° to 100° F) daily and transpiration was high.

For the results of these tests see table 2 and figure 3.

Conclusions.—If not overwatered, avocado plants can apparently remain in soil inoculated with *Phytophthora Cinnamomi* for at least 3 months without showing any ill effects; and when *Phytophthora Cinnamomi* was not present in the soil, the plants could be submerged for periods of 3, 6, or even 9 consecutive days without suffering any ill effects. But if this fungus is in the soil and the roots and soil are sub-

[9] Method from L. J. Klotz.

merged for 2 or 3 days (even 1 day is apparently sufficient), the plants are liable to attack; the roots turn black, and the plants rapidly wilt and die.

Plants in soil containing *Pythium vexans* showed no ill effects from 2 or 3 days' submersion.

Plants growing in a solution without aeration for a period of 3 weeks made little foliage or root growth, but otherwise appeared to remain normal. Those well-aerated produced new foliage and large numbers of roots. Zoöspores and mycelium of *Phytophthora Cinnamomi* added to plants growing in solution caused wilting and death, irrespective of whether the solution was aerated or not.

DISCUSSION

That avocado trees cannot stand excessive water at their roots appears to be recognized fact.[10] Dying-back, or decline, of the trees can generally be expected under such a condition, whether this be the result of faulty irrigation practice, heavy or continuous rains, a leak in a pipe line, or lack of drainage due to impervious subsoil fairly near the surface. Dying-back may occur even in sandy soils under excessively wet conditions. Many of the roots may be destroyed without apparently affecting the tree until several months later, when, with drier weather, the depleted stock of roots is unable to supply the tree adequately with nourishment, and dying-back becomes evident. The cause of the decline may then appear baffling, for at that time no sign of the excessive water conditions can be observed or would perhaps even be suspected.

The results of the present experiments may possibly throw some light on the problem. Too much water, alone, may not be the cause of the death of the roots, for it would seem that the fungus *Phytophthora Cinnamomi* plays an important part. The results of these experiments have confirmed those of earlier tests (*12, 13*) and show that this fungus does not attack the roots or affect the health of plants grown in soil where drainage is good and water is not excessive. But when the roots are allowed to stand in nonmoving water for even as short a period of time as 24 hours, they become susceptible to attack by the fungus; and the longer the period, the more drastic the results. On the other hand, roots immersed for 3 days in experiment 1 and for 9 days in experiment 2, in soil in which *Phytophthora Cinnamomi* was not present, were not affected at all. In fact, Horne (*7*) states that the roots of 2 healthy potted plants were immersed, and only after the eighteenth day did they wilt and subsequently die.

[10] This was emphasized by Dr. J. E. Coit in a lecture to the Avocado Department of the Los Angeles Farm Bureau at Whittier, California, February, 1940.

Apparently, avocado roots can stand immersion for certain lengths of time without harmful effects, unless *Phytophthora Cinnamomi* is present, when immersion, even for a short time, will cause injury. One may speculate that, with a lack of oxygen, cell activity and normal respiratory processes may cease, accumulations of substances in the cells may take place, or the outer layer cells of the root may be weakened or killed. Under moist conditions, zoöspores of the fungus would be produced. These zoöspores might be more virulent than the fungus mycelium in attacking the roots; but this hardly seems likely, for in a heavy soil saturated with water they would probably only be able to swim about on the soil surface. It remains to be determined in what manner the fungus brings about the injury. Dead roots of the wilted plants were found to be permeated with hyphae. If unfavorable conditions are not prolonged beyond a safe limit and if no fungus is present, the vital processes which have been slowed up or stopped in the cells apparently resume normal operation when normal soil conditions are restored, without injury to the roots.

Experiments showed that *Pythium vexans* was unable to attack and harm roots immersed for as long as 3 days. It is likely, therefore, that this fungus, although found frequently in the cultures, grows in weakened or dead roots, or in those attacked by *Phytophthora Cinnamomi.*

Pythium ultimum was found on only 1 root and was disregarded in this investigation.

No tests for the pathogenicity of *Fusarium oxysporum* and *Cylindrocarpon radicicola* were carried out, but it is suspected that they would prove no more harmful than *Pythium vexans.*

SUMMARY

Dying-back, or decline, of avocados in southern California appears to be commonly associated with excessive moisture.

Roots of affected trees are frequently blackened and dead, and the larger roots may have brown lesions on them. Two fungi, *Phytophthora Cinnamomi* Rands and *Pythium vexans* de Bary, were commonly isolated from such roots.

Phytophthora Cinnamomi had previously been recorded from avocados only in South Africa and in Puerto Rico.

In inoculation experiments it was found that if the plants were watered normally, *Phytophthora Cinnamomi* could be present in the soil for at least 6 months without affecting them seriously. If the roots of the plants and the soil were flooded or submerged for 2 or 3 days, or for but 24 hours, however, the fungus caused injury to the roots, followed by rapid wilting and subsequent death of the plants.

Control plants, in tests where no fungus was present, could withstand such flooding for as long as 9 days without suffering any subsequent harm.

The results of tests with *Pythium vexans* indicate that this fungus does not injure the roots but probably grows only in weakened or dead roots or in those already attacked by *Phytophthora Cinnamomi.*

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PYTHIACEOUS FUNGI ON CITRUS

VINCENT A. WAGER

PYTHIACEOUS FUNGI ON CITRUS[1,2]

VINCENT A. WAGER[3]

PYTHIACEOUS FUNGI on citrus in California were investigated during the season 1939–40. In the course of this investigation, citrus roots were examined for the presence of fungi, inoculation experiments were performed on citrus fruits with pythiaceous fungi from citrus in general, and growth-temperature relations of *Phytophthora* species found on citrus were studied. This paper reports the results of this work, and includes a compilation of records on the geographic distribution of *Phytophthora* on citrus and a description of all *Phytophthora* and *Pythium* species recorded on citrus.

PYTHIACEOUS FUNGI ON ROOTS OF CITRUS

In previously reported work on the isolation of fungi from roots of citrus, Fawcett (*3*)[4] states that species of *Pythium* and *Phytophthora* have been found to be associated with the damping-off and death of young citrus trees. Weindling (*11*) isolated *Phytophthora parasitica* Dastur and *Pythium* spp. from citrus seedlings affected with this disease in California, and Perlberger (*5*) found *Phytophthora citrophthora* (Sm. and Sm.) Leonian and *Phytophthora parasitica* in the same connection in Palestine. Fawcett (*1*) recorded the finding of *Phytophthora citrophthora* and *Phytophthora parasitica* in 1923 on large citrus roots and showed that the former would attack small roots of lemon trees. He (*2, 3*) also found *Phytophthora megasperma* Drechsl. on the fibrous roots of orange trees dying back in heavy clay soil in Tulare County, California. In 1935, Petri (*6*) found *Pythium megalacanthum* de Bary and *Pythium de Baryanum* Hesse associated with root rot of oranges in Catania, Italy.

In order to explore the possibility that species of *Phytophthora* or some other fungi might be playing a more active part in producing disease in citrus trees in California than had hitherto been suspected, large numbers of roots were examined from citrus trees that showed a

[1] Received for publication May 19, 1941.

[2] Paper No. 456, University of California Citrus Experiment Station, Riverside, California.

[3] Plant Pathologist, Union of South Africa Department of Agriculture. On Commonwealth Fellowship in collaboration with the Division of Plant Pathology, University of California Citrus Experiment Station, Riverside, California, September, 1939, to June, 1940.

[4] Italic numbers in parentheses refer to "Literature Cited" at the end of this paper.

dying-back, or decline. Orange and lemon trees growing in various localities in southern California were inspected.

Roots from the diseased trees were carefully washed in water; portions of the dead fibrous roots, about 1 cm long, were then surface-sterilized and placed on petri dishes poured with oatmeal agar. Culture tests were made from 320 fibrous roots of orange and 152 fibrous roots of lemon. The nonpythiaceous fungus *Fusarium Solani* (Mart.) App. and Wr.[5] was found on most of the roots from all localities.[6] Table 1 shows the pythiaceous fungi found and the frequency of their occurrence.

TABLE 1

Pythiaceous Fungi Isolated from Fibrous Roots of Citrus Trees Variously Located*

Fungus	Orange roots		Lemon roots	
	Number of localities where found	Number of roots infected, of 320 tested	Number of localities where found	Number of roots infected, of 152 tested
Phytophthora citrophthora	4	6	1	3
Phytophthora parasitica	1	1	0	0
Pythium de Baryanum	1	1	0	0
Pythium rostratum	1	1	0	0
Pythium ultimum	9	31†	3	7
Pythium vexans	2	3	1	2

* Root samples were from citrus trees in 22 different localities.
† Of these roots, 20 (of 24 tested) were from 1 locality.

Pythium ultimum Trow was the fungus most frequently found in these root samples, occurring on 38 out of 472 roots from 12 out of 22 localities. *Phytophthora citrophthora* was next, occurring on 9 roots from 5 localities. *Pythium vexans* de Bary was found on only 5 roots from 3 localities; and the other fungi (table 1) came from only 1 locality each. The absence of a given fungus from a few samples of roots from a

[5] Identified by W. C. Snyder, Assistant Professor of Plant Pathology and Assistant Plant Pathologist in the Experiment Station.

[6] Culture tests made from roots of citrus trees affected with a condition known as "dry root rot" have generally yielded *Fusarium Solani*. Attempts by various workers (*3*), however, to reproduce the disease by inoculation, with this fungus, of trees growing under healthy conditions, have yielded negative results.

To test the possible effect of *Fusarium* further, large numbers of young citrus trees, including some three-year-old trees growing in 5-gallon cans, were inoculated with the *Fusarium Solani* common in the more recent isolation tests by introducing the fungus, growing on sterilized wheat kernels, into the top layers of the soil without disturbing the roots. A month later, a number of these plants were submerged in larger containers of water for periods varying from 3 days to 3 weeks. They were then drained rapidly and were watered thereafter whenever necessary. During the following 6 months, none of these plants showed any ill effects from the presence of the fungus or from the period of submersion.

given tree is not much of an indication, however, that it was not to be found on that tree. In a study of fungi on avocado roots (*10*), *Pythium ultimum* was found on 1 root and *Pythium vexans* on 20.

INOCULATION OF CITRUS FRUIT WITH PYTHIACEOUS FUNGI

Inoculations were made on orange and lemon fruits with all the pythiaceous fungi recorded by Fawcett (*3*) and by Fawcett and Bitancourt (*4*), namely, *Phytophthora citrophthora* (Sm. and Sm.), *Phytophthora parasitica* Dastur, *Phytophthora palmivora* Butler, *Phytophthora Syringae* Kleb. (=*P. hibernalis* Carne), *Phytophthora cactorum* (L. and C.) Schroet. (=*P. citricola* Saw.), *Phytophthora megasperma,* and *Phytophthora Cinnamomi* Rands; and by Wager (*8, 9*), namely, *Pythium irregulare* Buis. from a rotting orange and *Pythium ultimum* Trow from the navel end of a young orange. These fungi included all the Pythiaceae previously obtained from citrus, with the exception of *Pythium megalacanthum* de Bary.

The relative importance of deep and shallow wounds (that is, those which penetrate the juice sacs and those which do not) in the production of rots caused by *Alternaria Citri* Ellis and Pierce and *Fusarium lateritium* Nees has been demonstrated (*9*). Accordingly, in these tests, inoculum (fungus growing on agar) was placed on the surface of the fruit and in shallow wounds, being covered in both cases with damp absorbent cotton; or it was placed in deep wounds made with a cork borer and sealed with vaseline. The results are presented in table 2.

Phytophthora citrophthora, P. parasitica, P. palmivora, and *P. cactorum* produced a brown rot of fruits, whether the inoculum was placed on the surface of the uninjured fruit or in shallow or deep wounds. The fruits inoculated with *P. Syringae* were kept at 18° C; there was no infection through uninjured epidermis, and the rot developed very slowly both in shallow and in deep wounds.

Phytophthora megasperma did not produce infection through uninjured epidermis, but did induce a slow, brown, leathery rot through shallow or deep wounds. *P. Cinnamomi* was also unable to pierce uninjured epidermis; through wounds, it produced a firm, brown, leathery rot, which was inclined to be of a drier type inside than that produced by the other species.

Pythium ultimum and *Pythium de Baryanum* were able, in a few cases, to infect through uninjured skin. Both of these fungi, through shallow or deep wounds, produced a brown rot and wrinkling of the skin, grew rapidly to the core, and traveled to both ends of the fruit,

TABLE 2

Infection of Citrus Fruits by Inoculation with Pythiaceous Fungi

Fungus, culture no., and source of culture	Number of fruits infected (of 3 inoculated) and rate of infection (R, rapid; S, slow)				
	Oranges		Lemons		
	Surface inoculation	Deep wound	Surface inoculation	Shallow wound	Deep wound
Phytophthora cactorum:					
2016,* from lemon fruit, Brazil	3 R	3 R	3 R	3 R	3 R
292,† from grapefruit, South Africa	3 S	3 S	2 S	3 S	2 S
Phytophthora Cinnamomi:					
2009,* from orange bark, Brazil	0	3 R	0	3 R	3 R
385,‡ from avocado root, South Africa	0	3 S	0	3 S	3 S
6, from avocado root, California	0	3 S	0	3 S	3 R
15, from avocado root, California	0	3 S	....	....	....
Phytophthora citrophthora:					
1309,* from lemon bark, California	3 R	3 R	3 R	3 R	3 R
3222,‡ from orange fruit, South Africa	3 R	3 R	3 R	3 R	3 R
29, from orange root, California	2 S	3 R	3 R	3 R	3 R
52, from lemon root, California	2 R	3 R	....	....	....
Phytophthora megasperma:					
1851,* from orange root, California	0	3 S	0	3 S	2 S
Phytophthora palmivora:					
2003,* from orange bark, Argentina	2 S	3 S	2 S	3 R	3 R
Phytophthora parasitica:					
2011,* from orange bark, Brazil	3 S	3 R	3 R	3 R	3 R
32,‡ from orange root, California	3 R	3 R	3 R	3 R	3 R
Phytophthora Syringae:					
1894,* from orange fruit, California	0	3 S	0	3 S	3 S
1839,* from orange fruit, California	0	3 S	0	3 S	3 S
Pythium de Baryanum:					
13, from orange root, California	0	3 R	1 R	3 R	3 R
Pythium irregulare:					
90, from orange fruit, South Africa	0	1 S	0	2 S	1 S
Pythium rostratum:					
37, from orange root, California	0	0	0	0	0
Pythium ultimum:					
1, from orange root, California	1 R	3 R	0	3 R	3 R
42, from lemon root, California	1 R	3 R	1 S	3 R	3 R
Pythium vexans:					
30, from orange root, California	0	3 S	0	3 S	3 S
38, from orange root, California	0	3 S	0	3 S	3 S

* Isolated by Fawcett.
† Isolated by Doidge.
‡ Isolated by Wager.

which then also showed infection. The decay was a much softer and slushier type than that produced by the *Phytophthora* species. *Pythium irregulare* behaved similarly, but was much less virulent and rotted only a few of the inoculated fruits. *Pythium vexans* produced a distinctive rot both in shallow and in deep wounds; it progressed slowly, developed a sunken, brown, slushy area with a water-soaked zone surrounding it,

TABLE 3

GROWTH-TEMPERATURE RELATIONS OF *Phytophthora* SPECIES

Fungus, culture no., and source of culture	Radial growth* of fungus after 4 days at 25° C and 4 days at different temperatures												
	1°	4°	7°	10°	13°	16°	19°	22°	25°	28°	31°	34°	37°
Phytophthora cactorum:	*mm*	*mm*	*mm*	*mm*	*mm*	*mm*	*mm*	*mm*	*mm*	*mm*	*mm*	*mm*	*mm*
2016,† from lemon fruit, Brazil	0	0	3	5.0	9.5	15.5	17.0	22.0	27.5	28.0	4.0	0.0	0
292,‡ from grapefruit, South Africa	0	0	4	10.0	15.0	22.0	27.5	26.5	24.0	23.5	5.0	1.5	0
Phytophthora Cinnamomi:													
2009,† from orange bark, Brazil	0	0	0	0.0	3.0	15.5	16.5	21.0	20.0	21.5	21.5	20.0	0
385,§ from avocado root, South Africa	0	0	0	2.5	11.0	22.5	30.5	38.0	41.0	37.0	22.5	2.0	0
Phytophthora citrophthora:													
1309,† from lemon bark, California	0	0	3	4.5	10.5	14.5	16.5	19.5	21.0	21.0	18.5	6.0	0
190,§ from grapefruit bark, South Africa	0	0	0	5.0	13.0	20.0	22.0	25.5	32.0	31.5	27.5	2.0	0
3222,§ from orange fruit, South Africa	0	0	0	5.5	13.5	19.0	27.0	29.0	28.5	25.0	20.0	2.0	0
Phytophthora megasperma:													
1851,† from orange root, California	0	2	8	12.0	16.5	20.0	22.5	19.5	18.5	17.5	2.0	0.0	0
Phytophthora palmivora:													
2003,† from orange bark, Argentina	0	0	0	0.0	3.5	8.5	17.0	22.0	25.0	23.5	23.5	17.0	4
Phytophthora parasitica:													
2011,† from orange bark, Brazil	0	0	0	0.0	4.0	18.0	22.5	24.5	29.0	30.0	32.0	27.0	5
Phytophthora Syringae:													
1894,† from orange fruit, California	0	2	3	5.0	5.5	5.5	1.0	0.0	0.0	0.0	0.0	0.0	0
1839,† from orange fruit, California	0	0	6	6.0	9.5	7.0	2.0	0.0	0.0	0.0	0.0	0.0	0
296,‡ from orange fruit, South Africa	0	0	4	8.0	11.0	11.0	1.0	0.0	0.0	0.0	0.0	0.0	0

* Average of three cultures. † Isolated by Fawcett. ‡ Isolated by Doidge. § Isolated by Wager.

and was soft and slushy inside. *Pythium rostratum* Butler and one strain of *Pythium vexans* (with coiled antheridial branch) did not produce any infection at all.

GROWTH-TEMPERATURE RELATIONS OF PHYTOPHTHORA SPECIES

The growth-temperature relations of *Phytophthora* species, based on radial growth of the mycelium in culture, are shown in table 3. The results agree with those of Fawcett and Bitancourt (*4*). *P. parasitica* has a slightly higher maximum than *P. citrophthora;* and *P. parasitica, P. palmivora,* and *P. Cinnamomi* from citrus in Brazil grew well at 34° C. *P. Syringae* is a low-temperature fungus, not growing at 22° or above and showing maximum growth between 13° and 16°. *P. megasperma* also has a low maximum, 19°.

DISTRIBUTION OF PHYTOPHTHORA SPECIES ON CITRUS

The world distribution of the *Phytophthora* species on citrus, as compiled from Fawcett's (*3*) records and from a survey of phytopathological literature up to 1940, is as follows:

Phytophthora cactorum:
- Argentina
- Brazil
- Japan
- South Africa

Phytophthora Cinnamomi:
- Brazil
- United States—California

Phytophthora citrophthora:
- Argentina
- Australia—New South Wales, Queensland, South Australia, Victoria, and West Australia
- Azores
- Belgian Congo
- Brazil
- Cyprus
- Egypt
- India
- Italy
- Japan
- Mexico
- Mozambique
- New Zealand
- Palestine
- Portugal
- Sicily
- South Africa
- Southern Rhodesia
- Spain
- United States—California and Florida
- West Indies, including Puerto Rico

Phytophthora megasperma:
- United States—California

Phytophthora palmivora:
- Argentina
- Ceylon
- East Indies, including Java
- India
- Malaya
- Philippine Islands
- Surinam
- Tanganyika Territory
- Uruguay
- West Indies, including Puerto Rico and Trinidad

Phytophthora parasitica:
- Argentina
- Australia—New South Wales
- Azores

Phytophthora parasitica (continued)
Brazil
Cuba
East Indies—Java
Italy
Japan
Mexico
Palestine
Paraguay
Philippine Islands
Portugal
Sicily
Spain
United States—California and Florida
Uruguay
West Indies, including the Lesser Antilles, Puerto Rico, and Trinidad

Phytophthora Syringae:
Australia—New South Wales, South Australia, Victoria, and West Australia
Azores
Portugal
South Africa
United States—California

IDENTIFICATION OF PYTHIACEOUS FUNGI ON CITRUS

For convenience in identifying Pythiaceae found on citrus, the characteristics of *Phytophthora* species are given in table 4 (see also fig. 1), and those of *Pythium* species in table 5 (see also fig. 2). All cultures described in these tables were examined by the writer except *Pythium megalacanthum.*

According to Tucker (7), the names *Phytophthora hibernalis* and *P. citricola* should be discarded in favor of *P. Syringae* and *P. cactorum,* respectively. Fawcett's cultures 1894 *P. Syringae* and 1839 *P. hibernalis* were found very similar in their cultural and morphological characters and in their reactions when inoculated into citrus fruits and are herein considered to be *P. Syringae.*

TABLE 4

MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERS OF *Phytophthora* SPECIES ON CITRUS

Fungus, culture no., and source of fungus	Characters of the isolates used in this study					Characters recorded in original description of species
	Distinguishing characters	Oögonia and oöspores	Antheridia	Chlamydospores	Sporangia	
Phytophthora cactorum (L. and C.) Schroet.:						
2016,* from lemon fruit, Brazil	Profuse production of oögonia with paragynous antheridia	Oögonia 18–42, commonly 30μ in diameter; oöspores about 6μ less; light yellow-brown	Paragynous	Only a few seen; 26μ in diameter; thin-walled, not colored	Papilla not prominent; 20–30 × 25–40μ in size; sparsely produced†	Oögonia average 26–29μ in diameter; chlamydospores very scarce; sporangia 25 × 32–42μ in size
292,‡ from grapefruit, South Africa	Profuse production of oögonia with paragynous antheridia	Oögonia 28–33, average 29.3μ in diameter	Paragynous	16–33, commonly 30μ in diameter	20–36 × 43–73μ, average 31 × 58μ in size	Oögonia average 26–29μ in diameter; chlamydospores very scarce; sporangia 25 × 32–42μ in size
Phytophthora Cinnamomi Rands:§						
2009,* from orange bark, Brazil	Production of chlamydospores in bunches	Oögonia 28–36, commonly 33μ in diameter; very rare in culture; oöspores 3–5μ less in diameter, with very thick wall	14–18μ in diameter; amphigynous; golden-brown	15–50, commonly 40μ in diameter; occurring in bunches; thin-walled	Ovoid; nonpapillate; 25–35 × 40–60μ, commonly 30 × 50μ in size; proliferous	Oögonia average diameter 32μ (Ashby) or 28μ (Tucker); chlamydospores 28–60, average 41μ in diameter; sporangia 18–43 × 25 100μ, average 33 × 57μ in size
Phytophthora citrophthora (Sm. and Sm.) Leonian:						
1309,* from lemon bark, California	Absence of oögonia; optimum growth at 25–27.5° C, none at 32.5°	Not found	Not found	Spherical; thin-walled; 20–40, commonly 30–35μ in diameter	Prominently papillate; 12–40 × 20–50μ, commonly 25 × 40μ in size	Oögonia unknown; chlamydospores commonly 28μ in diameter; sporangia 20–60 × 30–90μ, average 35 × 50μ in size; may retain a pedicel
190,¶ from grapefruit bark, South Africa	Absence of oögonia; optimum growth at 25–27.5° C, none at 32.5°	Not found	Not found	Very rare; 25–30μ in diameter	20–40 × 23–56μ, average 35 × 41μ in size	Oögonia unknown; chlamydospores commonly 28μ in diameter; sporangia 20–60 × 30–90μ, average 35 × 50μ in size; may retain a pedicel
Phytophthora megasperma Drechsl.:						
1851,* from orange root, California	Large size of oögonia and absence of chlamydospores	Oögonia 30–54, average 43μ in diameter; oöspores about 6μ less, with thick yellow wall	12–15μ in diameter; usually paragynous but may be amphigynous	Not found	Ovoid or sometimes papillate; 25–42 × 35–54μ, commonly 35 × 46μ in size; proliferous	Oögonia 16–61, average 47.4μ in diameter; sporangia 6–45 × 15–90μ in size

TABLE 4—(*Continued*)

Phytophthora palmivora Butler: 2003,* from orange bark, Argentina	Absence of oögonia; optimum growth at 27.5–30° C; will grow at 32.5°	Not found	Not found	Produced in abundance; spherical; may be intercalar; 25–40, commonly 30μ in diameter; thin-walled	Prominently papillate; 22–35 × 35–55μ, commonly 32 × 40μ in size	Oögonia produced only in paired cultures; oöspores 22–24μ in diameter; antheridia amphigynous; chlamydospores 32–42μ in diameter; sporangia 25–35 × 40–60μ in size, with short, stout pedicel
Phytophthora parasitica Dastur: 2011,* from orange bark, Brazil	Optimum and maximum growth temperatures about 3° higher than that for *P. citrophthora*	Oögonia 27–36, commonly 32μ in diameter; oöspores 3–5μ less, thick-walled, yellow-brown	Mainly amphigynous	Produced in abundance; 27–42, commonly 30μ in diameter; thin- or thick-walled; may be yellow	Prominently papillate; 15–30 × 18–48μ, commonly 28 × 40μ in size	Oögonia group microspora, 12–24μ in diameter, average under 20μ; macrospora 20–35μ in diameter, average over 20μ; chlamydospore ±30μ in diameter; sporangia average more than 25 × 30μ in size
32,¶ from orange root, California	Optimum and maximum growth temperatures about 3° higher than that for *P. citrophthora*	Oögonia 24–33, commonly 30μ in diameter		28–54, commonly 30μ in diameter	21–45 × 24–60μ, commonly 35 × 48μ in size	Oögonia group microspora, 12–24μ in diameter, average under 20μ; macrospora 20–35μ in diameter, average over 20μ; chlamydospore ±30μ in diameter; sporangia average more than 25 × 30μ in size
Phytophthora Syringae Kleb.: 1894,* from orange fruit, California	Grows at low temperatures; optimum growth at 13–16° C	Oögonia 27–36, commonly 32μ in diameter; oöspores 3–5μ less, thick-walled, light yellow	Small, 9–12μ in diameter; paragynous	Not found	Papilla flattened or protruding but without hyaline plug; 15–18 × 24–42μ, commonly 18 × 36μ in size; thin persistent pedicel 10–25μ long	Oögonia, average diameter 28.4μ (Tucker), 40.8μ (Carne); sporangia 15–33 × 28–41μ, average 17.9 × 34.4μ (Tucker), 16.1 × 34.6μ (Carne)
296,‡ from orange fruit, South Africa	Grows at low temperatures; optimum growth at 13–16° C	Oögonia 26–40, commonly 35.2μ in diameter		Not found	15–25 × 26–57μ, commonly 20 × 37.5μ in size	Oögonia, average diameter 28.4μ (Tucker), 40.8μ (Carne); sporangia 15–33 × 28–41μ, average 17.9 × 34.4μ (Tucker), 16.1 × 34.6μ (Carne)

* Isolated by Fawcett.

† In one test with *Phytophthora cactorum*, a culture on oatmeal agar was flooded with pea broth, and sporangia were produced in abundance, very irregular in shape, mostly elongated, and very variable in size (45–90, commonly 60 x 30μ).

‡ Isolated by Doidge.

§ This fungus from citrus was compared with *Phytophthora Cinnamomi* from roots of avocado from South Africa and from California. All were found to be very similar morphologically and all produced oögonia on oatmeal-agar tubes that had been kept in the laboratory over winter and were 3 to 6 months old.

¶ Isolated by Wager.

TABLE 5

MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERS OF *Pythium* SPECIES ON CITRUS

Fungus and source of culture	Characters of isolates used in this study			Characters recorded in original description of species*
	Oögonia and oöspores	Antheridia	Sporangia	
Pythium de Baryanum Hesse, from orange root, †‡ California	Oögonia 16–26, commonly 20μ in diameter; usually terminal; may be intercalar; oöspores about 3μ less in diameter	Commonly 1 or 2 per oögonium, may be more; arise on same hypha as, and some distance from, the oögonium or on separate hypha	14–28, commonly 22μ in diameter; terminal and spherical or may be intercalar and oval; thin-walled; production of zoöspores not observed	Oögonia 15–28, average 21μ in diameter; sporangia 15–26, average 19μ in diameter; zoöspores produced
Pythium irregulare Buis., from orange fruit, ‡ South Africa	Oögonia 16–24, commonly 22μ in diameter; may be terminal; mostly intercalar; spherical, oval, irregularly lobed, or with few blunt, digitate spines; oöspores about 3μ less in diameter	Commonly 2 or 3 per oögonium; clavate and crook-necked, usually with fairly long stalk arising from same or neighboring hypha	13–28μ in diameter; very variable in shape: terminal and spherical or may be intercalar and elliptic or irregular in shape; evacuation tube usually ⅓ to ½ length of oögonium	Oögonia 16–18μ in diameter; sporangia 10–20μ in diameter; zoöspores produced
Pythium megalacanthum de Bary, § from orange root, ¶ Italy	Oögonia 36–45μ in diameter, exclusive of spines; terminal or intercalar; spines 6–9μ long, conical, acutely tipped; oöspores smooth	One or more per oögonium; diclinous	Terminal or intercalar; spherical to subspherical; frequently proliferous, forming a second sporangium above the primary; zoöspores produced	Oögonia 42–54μ in diameter; sporangia frequently proliferous; zoöspores produced
Pythium rostratum Butler, from orange root, †‡ California	Oögonia 12–27, commonly 21μ in diameter; occasionally terminal; usually intercalar; may occur in chains; oöspores usually filling oögonia	Usually 1, rarely 2 or 3 per oögonium; may arise at base of oögonium or may be a portion of oögonial stalk; sometimes swollen	15–27, commonly 24μ in diameter; usually spherical; may be intercalar and oval, or barrel-shaped; thin-walled; production of zoöspores not observed	Oögonia usually 21μ in diameter and intercalar; sporangia 23–34, average 28μ in diameter; zoöspores produced
Pythium ultimum Trow, from orange root, †‡ California	Oögonia 18–24, commonly 21μ in diameter; usually terminal and spherical; may be intercalar; oöspores about 3μ less in diameter	Usually 1, curved, arising immediately below the oögonium; usually sessile or from a different hypha	16–26, commonly 21μ in diameter; usually terminal and spherical; may be intercalar, thin-walled	Oögonia 19.6–22.9, average 20.6μ in diameter; sporangia 12–28, average 20μ in diameter; zoöspores not produced
Pythium vexans de Bary, from orange root, †‡‖ California	Oögonia 15–24, commonly 20μ in diameter; mostly terminal; few intercalar; oöspores about 3μ less in diameter	Usually 1, rarely 2 per oögonium; swollen, clavate or bell-shaped, usually on long branch arising from hypha bearing oögonium	12–26, commonly 22μ in diameter; usually terminal and spherical; may be intercalar and elliptic or irregular in shape; evacuation tube usually ⅓ to ½ length of oögonium	Oögonia 15–28, average 22μ in diameter; sporangia 17–24, average 21μ in diameter; zoöspores produced

* Middleton, John T. Taxonomy of the genus *Pythium* Pringsheim. Thesis in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of Missouri, 1940. (Typewritten.) Copy on file in the Library of the University of Missouri, Columbia.

† Fibrous roots.

‡ Isolated by Wager.

§ This fungus was not seen by the writer; the description given is that of its author, de Bary.

¶ Isolated by Petri.

‖ One fungus isolated from lemon rootlets differed from the typical *P. vexans* in having a coiled antheridial branch and mostly irregular-shaped sporangia, as shown in fig. 2, *D*, *E*, and *H*. Zoöspores were produced in the same manner as those of other cultures of *P. vexans* and measurements of oögonia and sporangia were not materially different. Middleton considers this fungus a strain of *P. vexans*.

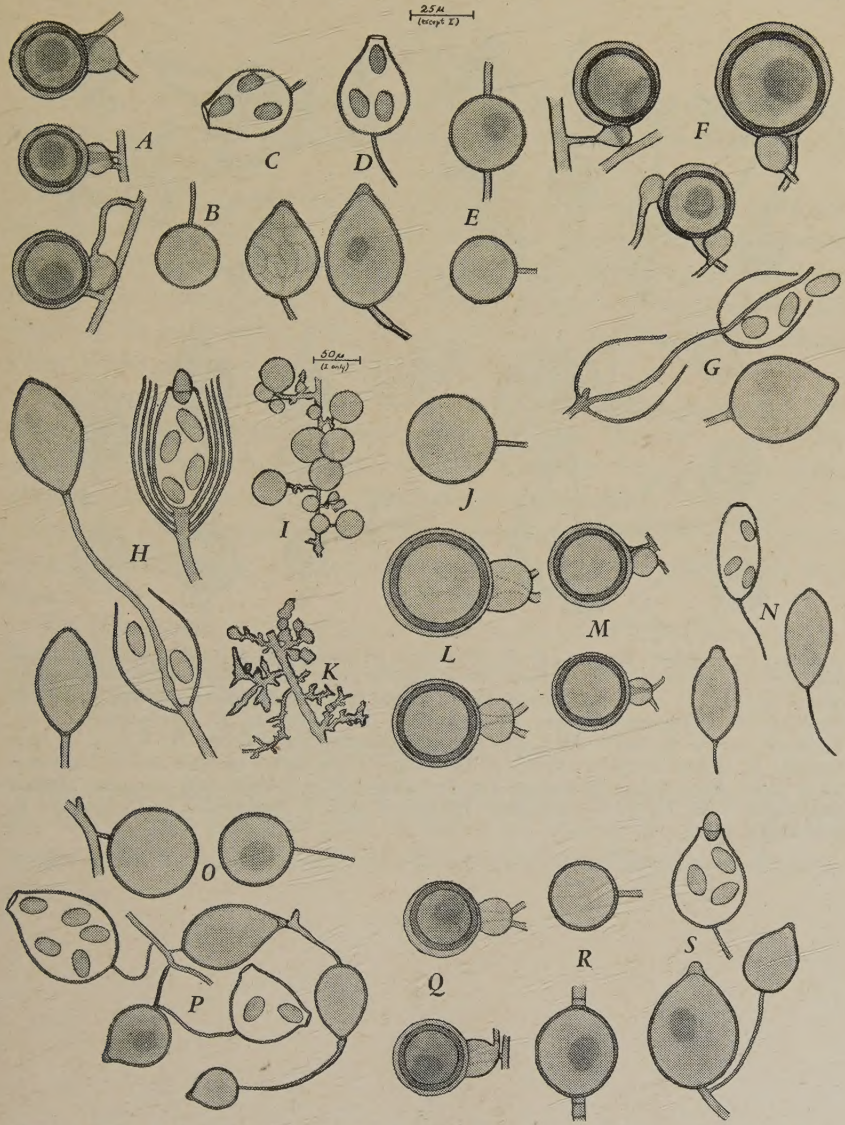

Fig. 1.—*Phytophthora* species. *A–C, Phytophthora cactorum* (L. and C.) Schroet.: *A,* oögonia and paragynous antheridia; *B,* chlamydospore; *C,* sporangia. *D–E, Phytophthora palmivora* Butler: *D,* sporangia; *E,* chlamydospores. *F–G, Phytopthora megasperma* Drechsl.: *F,* oögonia and paragynous antheridia; *G, sporangia. H–L, Phytophthora Cinnamomi* Rands: *H,* sporangia; *I, J,* chlamydospores; *K,* mycelium; *L,* oögonia and amphigynous antheridia. *M–N, Phytophthora Syringae* Kleb.: *M,* oögonia and paragynous antheridia; *N,* sporangia with persistent pedicels. *O–P, Phytophthora citrophthora* (Sm. and Sm.) Leonian: *O,* chlamydospores; *P,* sporangia. *Q–S, Phytophthora* parasitica Dastur: *Q,* oögonia and amphigynous antheridia; *R,* chlamydospores; *S,* sporangia.

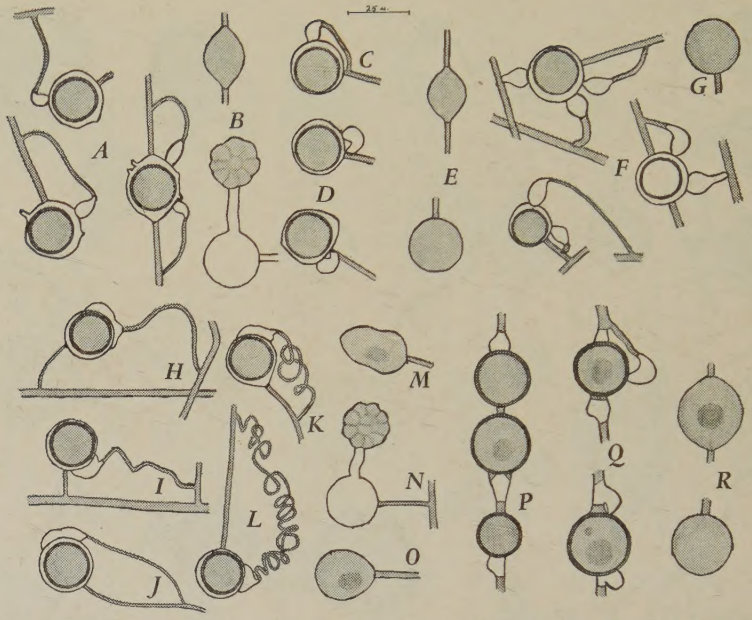

Fig. 2.—*Pythium* species. *A–B, Pythium irregulare* Buis.: *A,* antheridia and oögonia; *B,* sporangia. *C–E, Pythium ultimum* Trow: *C,* stalked antheridium that occurs rarely; *D,* common type of antheridium and oögonium; *E,* sporangia. *F–G, Pythium de Baryanum* Hesse: *F,* antheridia and oögonia; *G,* sporangium. *H–O, Pythium vexans* de Bary: *H, I, J, K, L,* oögonia and antheridia; *M, N,* and *O,* sporangia; *K, L,* and *M* are of a strain of the fungus from lemon rootlets and differ somewhat from other forms in having a coiled antheridial branch and irregular-shaped sporangia. *P–R, Pythium rostratum* Butler: *P* and *Q,* antheridia and oögonia; *R,* sporangia.

SUMMARY

Cultures were made from fibrous dead roots of orange and lemon trees growing in various localities in southern California and showing a dying-back, or decline.

Pythium ultimum, Pythium de Baryanum, Pythium vexans, Pythium rostratum, Phytophthora citrophthora, and *Phytophthora parasitica* were found on some of these roots, *Pythium ultimum* being the most frequent. The occurrence of the last-named fungus was very infrequent, however, in comparison with that of the nonpythiaceous fungus *Fusarium Solani,* which was found on almost every root.

The results of inoculation tests on orange and lemon fruits with the aforementioned *Pythium* species, with *Pythium irregulare,* and with all the *Phytophthora* species that have been isolated from citrus, namely, *Phytophthora citrophthora, Phytophthora parasitica, Phytophthora palmivora, Phytophthora Syringae, Phytophthora cactorum, Phytophthora Cinnamomi,* and *Phytophthora megasperma* are reported in this paper.

The distribution of the *Phytophthora* species and descriptions of the morphological characters of the *Phytophthora* and *Pythium* species which have been recorded on citrus are given.

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